Ministry of Economic Development
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PCT/NZ03/00184

CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 20 August 2002 with an application for Letters Patent number 520896 made by Glycox Corporation Limited.

I further certify that pursuant to a claim under Section 24(1) of the PatentsAct 1953, a direction was given that the application proceed in the name of PROTEMIX CORPORATION LIMITED.

Dated 10 September 2003.

PRIORITY DOCUMENT
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NEW ZEALAND

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SUBSTITUTION OF APPLICANT
UNDER SECTION 24

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

"Dosage Forms and Related Therapies"

We, GLYCOX CORPORATION LIMITED, a company duly incorporated under the laws of New Zealand of Level 4, 41 Shortland Street, Auckland, New Zealand, do hereby declare this invention to be described in the following statement:

FIELD OF THE INVENTION

The present invention relates to dosage forms and therapies and more particularly (but not solely) to methods of

(A) reversing in a patient

(I) being a diabetic human being or other diabetic mammal or

(II) a human being or other mammal with copper levels capable of diminishment ("the patient")

cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences) and/or

coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences), and/or

(B) improving by reversal of damage in a patient

(I) being a diabetic human being or other diabetic mammal or

(II) being a human being or other mammal with copper levels capable of diminishment

any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, magnetic resonance imaging (MRI) or other imagining), and/or

(C) reversing in a patient

(I) being a diabetic human being or other diabetic mammal or

(II) being a human being or other mammal with copper levels capable of diminishment ("the patient").

disorders or the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood

vessels (and the consequences thereof),

reliant upon, as active ingredient(s), trientine (See Martindale 33rd edition, 1025.3), salts of trientine and/or metabolites thereof.

The invention also consists in methods of reversing in a patient at least some of any damage arising from diabetic kidney disease, diabetic nephropathy and/or copper

accumulation in the kidney, and/or reversing in a patient at least some of any damage to the renal arteries reliant upon the abovementioned active ingredient(s).

The patient may have elevated copper levels.

Included in the present invention are dosage forms of the active ingredient(s) and relates to related uses (including uses in the preparation of pharmaceutical compositions of trientine, salts of trientine and/or metabolites thereof). Such dosage forms are indicated for at least both diabetic and elevated copper level patients.

By way of background the following can be stated in respect of diabetic heart disease in the human being,

1. Worldwide prevalence of diabetes is increasing. Number of cases of type 2 diabetes projected to increase from 135 million in 2000 to more than 300 million in 2025. Increase is related to ageing of the population, increasing obesity, and low socio-economic status. See, WHO. The World Health Report 1997.

2. Mortality from diabetes has increased over the last decade whereas mortality from cardiovascular disease, stroke, and malignant diseases has remained static or declined. See, US Centre for Health Studies.

3. Causes of premature mortality in type 2 diabetes comprise cardiovascular disease, 58%; cerebrovascular disease, 12%; nephropathy, 3%; diabetic coma, 1%; malignancy, 11%; and infections 4%. See, Pickup J, Williams G eds. Handbook of diabetes, 2nd edition, 1999; p 24.

4. Diabetic heart disease is characterised by more severe coronary artery disease at a younger age, a 4-fold increased frequency of heart failure post-acute myocardial infarction and a disproportionate increase in left ventricular hypertrophy. See Struthers AD, Morris Ad, Lancet 2002;359:1430-2.

5. Patients with type 2 diabetes manifest a disproportionate increase in mortality within the first 24-hours post-acute myocardial infarction. Acute intervention can ameliorate this risk. See, Malmberg K Br Med J 1997;314:1512-5.

Much of the above is equally applicable to diabetic coronary artery structure.

PCT/NZ99/00161 (published as WO00/18392 on 6 April 2000) has disclosed a

method of treating a mammalian patient predisposed to and/or suffering from diabetes mellitus with a view to minimising the consequences of macrovascular and microvascular damage to the patent which comprises, in addition to any treatment in order to control blood glucose levels, at least periodically inhibiting or antagonizing fructosamine oxidase enzyme activity in the patient. An assay for such activity is disclosed in their PCT/NZ99/00160 (published as WO00/18891 on 6 April 2000).

A range of different agents capable of acting as fructosamine oxidase inhibitors and/or antagonists were disclosed in PCT/NZ99/00161. These included copper chelating agents, substrate analogues and hydrazine compounds.

The full contents of the aforementioned specifications are here included by way of reference.

We have hypothesised that reduction in available free copper does have an affect in preventing macrovascular, microvascular and/or toxic/metabolic diseases of the kind hereinafter exemplified and in tissue repair processes. This is irrespective of the glucose metabolism of the patient.

We have also hypothesized that cardiovascular accumulation of redox-active transition metal ions is responsible for many of the adverse outcomes in diabetes. Under physiological conditions, injury to a target organ is sensed by distant stem cells, which migrate to the site of damage then undergo alternate stem cell differentiation; these events promote structural and functional repair. However, the accumulation of redox-active transition metals, particularly copper in cardiac or vascular tissues in subjects with diabetes is accompanied by a suppression of the normal tissue regeneration effected by the migration of stem cells. Elevated tissue levels of copper suppress these normal biological behaviours of such undifferentiated cells. Conditions occurring in the context of diabetes or impaired glucose tolerance, in which the suppression of normal stem cell responses can cause impairment of normal tissue responses, include the following:

1. Cardiac failure
2. Acute myocardial infarction
3. Wound healing and ulceration
4. Tissue damage caused by infection

5. Diabetic kidney damage

Conditions in which therapy to lower copper values in diabetic patients (ie; with IGT or Type 2 Diabetes Mellitus) is liable to prove beneficial include at least the following:

1. HEART FAILURE IN THE CONTEXT OF DIABETES

Significant regeneration of cardiac tissues can occur within a few days of cardiac transplantation. The likely mechanism is migration of stem cells from extra-cardiac sites to the heart, with subsequent differentiation of such cells into various specialized cardiac cells, including myocardial, endothelial and coronary vascular cells. We believe that copper accumulation in cardiac tissues is likely to severely impair these regenerative responses. Hence a role for acute intravenous therapy with a copper chelator in the treatment of diabetic heart failure.

2. MYOCARDIAL INFARCTION IN THE CONTEXT OF DIABETES.

Myocardial infarction is accompanied by proliferation of cells in the ventricular myocardium when MI occurs in the context of diabetes, the presence of elevated tissue levels of redox-active transition metals suppresses normal stem cell responses, resulting in impaired structural and functional repair of damaged tissues. Up to 20% of cells in the heart may be replaced by stem cell migration from extra-ventricular sites, as soon as four days after cardiac transplantation. These observations suggest that treatment of AMI in the context of diabetes will be improved by acute (if necessary, parenteral) as well as by subsequent chronic administration of chelators. The mechanism of the impairment of cardiac function in diabetes is likely a toxic effect of accumulated transition metals on tissue dynamics, resulting in impaired tissue regeneration caused in turn by suppression of normal stem cell responses, which mediate physiological tissue regeneration by migration to damaged tissue from external sites.

3. WOUND HEALING AND ULCERATION IN THE CONTEXT OF DIABETES

The processes of normal tissue repair require intervention of mobilizing stem cells, which effect repair of the various layers of blood vessels, for example. We believe that an accumulation

of transition metals (particularly copper) in vascular tissues causes the impaired tissue behaviour characteristic of diabetes, including impaired wound repair following surgery or trauma, and the exaggerated tendency to ulceration and poor healing of established ulcers. We believe that the treatment of diabetics with copper chelators before they undergo surgery, or in the context of traumatic tissue damage, is likely to be of benefit. It is probable that surgery in diabetics would have a better outcome if excess transition metals were removed from blood vessels prior to surgery. This may need to be accomplished on either an acute basis (with parenteral therapy) or on a more chronic basis (with oral therapy) prior to actual surgery.

4. SOFT TISSUE DAMAGE RESULTING FROM INFECTION AND OCCURING IN THE CONTEXT OF DIABETES OR IMPAIRED GLUCOSE TOLERANCE

We believe the processes of normal tissue repair following infection require intervention of mobilized stem cells, which migrate to sites of tissue damage to effect tissue regeneration and repair, for example, of the various layers of blood vessels. Such tissue damage will be impaired by suppressed stem cell responses, such as those caused by the build up of redox-active transition metals (particularly copper) in tissues, for examples the walls of blood vessels.

5. KIDNEY DAMAGE OCCURING IN THE CONTEXT OF DIABETES

We believe that impaired stem cell responses in the kidneys of diabetics contribute to diabetic nephropathy and renal failure. We believe that treatment of diabetics having kidney failure by administration of a copper chelator will improve organ regeneration by restoring normal tissue healing by allowing stem cells to migrate and differentiate normally.

However, even in the non diabetic mammal and even in a mammal without a glucose mechanism abnormality, we have hypothesised a reduction in extra-cellular copper values is advantageous in that such lower levels will lead to one or both a reduction in copper mediated tissue damage and improved tissue repair by restoration of normal tissue stem cell responses.

In our own studies (using the streptozocin-diabetic (STZ) rat model) we have found a high frequency of tissue damage in the heart tissue and coronary artery tissue in severely diabetic animals. This reflects what is found in man.

We now more firmly take the view that copper values (and particularly copper II) not bound internally of cells is available to mediate (together with available reducing substances) the generation of damaging free radicals that have a role in both tissue damage and impairment of stem cell mediated repair of such tissue. This is irrespective of diabetic status but we believe is more prevalent in diabetic rats and other mammals including human beings.

In respect of such damage and repair impairment we propose a diminishment in available free copper values as being an appropriate approach for any at risk patient (whether diabetic patients or any other patient (particularly a patient not suffering Wilson Disease) who has copper levels capable of diminishment).

Our agent of choice is trientine (ie TETA), preferably as an acid addition salt.

Alternative names for trientine include N,N'-Bis(2-aminoethyl)-1,2-ethanedi-amine; triethylenetetramine ("TETA"); 1,8-diamino-3,6-diazaoctane; 3,6-diazaoctane-1,8-diamine; 1,4,7,10-tetraazadecane; trien; TETA; TECZA and triene.

Reference made herein to "trientine" refers to TETA. Preferably the trientine is rendered less basic (eg; as a moiety in a delivery form) but can include analogues thereof which are prodrugs of the active copper chelating moiety or a copper chelating metabolite of trientine. Salts of trientine (which optionally can be salts of a prodrug of the trientine copper chelating moiety or metabolite) are preferably acid addition salts such as, for example, those of suitable mineral or organic acids.

Salts of trientine (such as acid addition salts, eg; trientine dihydrochloride) act as copper-chelating agents, which aids the elimination of copper from the body by forming a stable soluble complex that is readily excreted by the kidney.

Trientine, a strongly basic moiety, with its multiple nitrogens can be converted into a large number of suitable associated acid addition salts using an acid, for example, by reaction of stoichiometrically equivalent amounts of trietine and of the acid in an inert solvent such as ethanol or water and subsequent evaporation if the dosage form is best formulated from a dry salt. Possible acids for this reaction are in particular those which yield physiologically acceptable salts. Thus inorganic acids can be used, e.g. sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid,

phosphoric acids such as orthophosphoric acid, sulfamic acid. Furthermore organic acids, can be used, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic mono- or polybasic carboxylic, sulfonic or sulfuric acids, (e.g. formic acid, acetic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acid, nicotinic acid, isonicotinic acid, methane-or ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono-and-disulfonic acids, and laurylsulfuric acid).

The trientine moieties can also be in the form of quarternary ammonium salts in which the nitrogen atom carries a suitable organic group such as an alkyl, alkenyl, alkynyl or aralkyl moiety.

Preferably the trientine moieties are in the form of a compound or buffered in solution and/or suspension to a near neutral pH much lower than the pH of 14 of trientine itself.

Suitable anions may include citrate, isocitrate, α-Ketoglutarate, Succinate, Fumarate, Malate, Oxaloacetate, Acetate and pyruvate.

Trientine moieties (preferably delivered as a salts of trientine (such as acid addition salts, eg; trientine dihydrochloride) act as copper-chelating agents, which aids the elimination of copper from the body by forming a stable soluble complex that is readily excreted by the kidney.

The presumed site of action of the chelating trientine moiety of a salt such as trientine dihydrochloride is the removal of loosely bound copper from the body and in particular from the cardiac extracellular matrix and the coronary extracellular matrix. Bioavailabilities of the active species of trientine dihydrochloride after oral administration is low (<10%) due to poor absorption and marked first-pass metabolism. Trientine dihydrochloride and its transformed metabolite, N-acetyl-trientine hydrochloride, are both capable of binding copper, although the chelating activity of the analogue N-acetyl-trientine hydrochloride is significantly lower than trientine dihydrochloride. See, Kodama H. Life Sciences 1997;61:899-907.

Absorption of trientine dihydrochloride is adversely affected by food, mineral supplements and other drugs.

We have now shown in the STZ rat model for both diabetic man and non diabetic man a diminishment in available free copper does have an affect in reversing both

(i) cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extra cellular space and increased deposition of extra cellular matrix (and its consequences) and

(ii) coronary artery structure damage (and its consequences).

In so showing reversal of damage in the STZ rat, we have found a dose relativity for man insofar as the copper scavenging into the urine is concerned.

Under physiological conditions we believe injury to the cardiac structure is sensed by distant stem cells, which migrate to the site of damage then undergo alternate stem cell differentiation; these events promote structural and functional repair. However, the accumulation of redox-active transition metals, particularly copper in cardiac tissues and coronary arteries in subjects with diabetes we believe is accompanied by a suppression of the normal tissue regeneration effected by the migration of stem cells. Elevated tissue levels of copper suppress these normal biological behaviours of such undifferentiated cells.

Even in the non diabetic mammal (e.g. without Type 2 Diabetes mellitus) and even in a mammal without a glucose mechanism abnormality (e.g. without IGT or without IFG), we believe a reduction in extra-cellular copper values is advantageous in that such lower levels will lead to one or both a reduction in and a reversal of eg; by what we believe to be improved tissue repair by restoration of normal tissue stem cell responses.

OBJECT OF THE INVENTION

It is an object of the present invention to provide sustained, controlled and/or extended release dosage forms useful for taking advantage of this prospect for the purpose of reduction of such structure damage and damage reversal all irrespective of whether or not our hypothesis or proposals as to mode of action are correct.

Such damage includes cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition

of extracellular matrix (and its consequences) and/or coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences), whether or not the hypotheses or proposals as to mode of action are correct.

It is another object to provide uses and dosage forms applicable instead or as well to improve by reversal of damage in a human being or other mammal (preferably diabetic and/or with a copper level capable of chelation diminishment) ("the patient") any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imaging), whether or not the hypotheses or proposals as to mode of action are correct.

It is another object of the present invention to provide methods of treatment and related methods, uses and pharmaceutical compositions that reverse damage arising from any one or more disease states of the cardiovascular tree (including the heart) and dependent organs (eg; retina, kidney, nerves, etc.) exacerbated by elevated non-intracellar free copper values levels, whether or not the hypotheses or proposals as to mode of action are correct.

Reference herein to diseases of the cardiovascular tree and diseases of dependent organs includes any one or more of

(i) disorders of the heart muscle (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (macrovascular disease) such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (microvascular disease) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) plaque rupture of atheromatous lesions of major blood vessels such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries and the popliteal arteries.

The present invention relates to any such ailments and their treatment irrespective (unless otherwise stated) of any diabetic and/or glucose abnormality state of the mammalian patient.

Controlled drug delivery devices containing trientine or salts thereof

The present invention also is directed to novel formulations of TETA or salts thereof, useful for the pharmacological therapy of diseases in humans and other mammals, particularly those suffering from one or more of the following conditions in which elevated tissue copper plays an important role in disease initiation or progression: heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, Wilson's disease, or any form of cancer. The use of these controlled delivery preparations of trientine enables effective treatment of these conditions, through novel and improved formulations of the drug suitable for administration to humans and other mammals.

Controlled drug delivery formulations

The objective of controlled delivery formulations of TETA is to optimise its bioavailability and to maintain plasma concentrations within the therapeutic range for extended periods. Effective controlled drug delivery results in increases in the time that trientine plasma concentrations remain within the therapeutic range. Controlled delivery preparations also minimize the wide variations in drug concentration at the site of action achieved by conventional formulations, which frequently fail to achieve true "steady

state" levels, and thereby minimize periods of under and over medication. Such difficulties are especially pronounced for drugs of short biological half-life. In order to achieve a suitable kinetic profile for trientine, it will be necessary to modify conventional dosage forms in order to provide for increased utility of the drug, for application in indications such as cardiovascular disease, Wilson's disease, or oncology. Examples of controlled drug formulations can be found in standard references (for example, see: Sweetman, S. C. (Ed.). Martindale. The Complete Drug Reference, 33rd Edition, Pharmaceutical Press, Chicago, 2002, 2483 pp.; see also: Aulton, M. E. (Ed.) Pharmaceutics. The Science of Dosage Form Design. Churchill Livingstone, Edinburgh, 2000, 734 pp.; see also: Ansel, H. C., Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, 676 pp.). Excipients employed in the manufacture of drug delivery systems are described in various publications known to those skilled in the art (for example, see: Kibbe, E. H. Handbook of Pharmaceutical Excipients, 3rd Ed., American Pharmaceutical Association, Washington, 2000, 665 pp.). The USP provides many examples of modified-release oral dosage forms, including those formulated as tablets or capsules (for example, see: The United States Pharmacopeia 23/National Formulary 18, The United States Pharmacopeial Convention, Inc., Rockville MD, 1995). This publication also presents general chapters and specific tests to determine the drug release capabilities of extended-release and delayed-release tablets and capsules.

The regulatory compliance of trientine-containing extended-release and delayed-release preparations may be determined by tests described in the USP (supra). The USP test for drug release for extended-release and delayed-release articles is based on drug dissolution from the dosage unit against elapsed test time. Descriptions of the various test apparatus and procedures may be found in the USP, Chapter <724> (supra). The individual monographs contain specific criteria for compliance with the test and the apparatus and test procedures to be used. Examples have been given, for example for the release of aspirin from Aspirin Extended-release Tablets (for example, see: Ansel, H. C., Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, p. 237). Modified-release tablets and capsules must

meet the USP standard for uniformity as described for conventional dosage units. Uniformity of dosage units may be demonstrated by either of two methods, weight variation or content uniformity, as described in USP Chapter <905> (supra). Further guidance concerning the analysis of extended release dosage forms has been provided by the F.D.A. (see: Guidance for Industry. Extended release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations. Rockville, MD: Center for Drug Evaluation and Research, Food and Drug Administration, 1997).

Compliance of a dosage regime is always essential in order to derive the best benefit from a treatment regime. The present invention recognises a benefit from sustained release dosage forms that can provide such levels of sustained delivery to a patient as are required to elicit the advantages now seen from the prospect of lower overall dose delivery of trientine formulations when one compares them to the QID (four times) a day multiple dosage oral regimes hitherto used with trientine formulations for Wilson's disease.

Types of drug delivery systems

Modified-release (MR) dosage forms of the present invention include those listed in this paragraph, and which are defined below. Over the years, many terms (and abbreviations) have been employed by pharmaceutical manufacturers to describe product types and features. Widely used terms and abbreviations include: delayed-release (DR); sustained-release (SR); sustained-action (SA); prolonged-action (PA); controlled-release (CR); extended-release (ER); timed-release (TR); and long-acting (LA). For the most part, these terms are used to describe orally administered dosage forms, whereas the term rate-controlled delivery is applied to certain types of drug delivery systems in which the rate of drug delivery is controlled by features of the device rather than by physiological or environmental conditions such as gastrointestinal pH or drug transit time through the gastrointestinal tract. DR formulations effect delayed total drug release form some time after drug administration. RA formulations effect drug release in small aliquots intermittently after administration. SR formulations effect drug release slowly at a controlled rate governed by the delivery system. CR formulations effect drug release at a

constant rate that does not vary. ER formulations effect drug release for a significantly longer period than usual formulations.

The advantages of such formulations are as follows: convenience to the patient; increased compliance and achievement of steady state drug levels with twice-daily (b.d.) dosing; smoothening of plasma drug profiles to a constant level for extended time periods; prevention of drug toxicity; and elimination of breakthrough of therapeutic failure, especially at night.

Modified-release dosage forms: This term describes dosage forms having drug release features based on time, course, and/or location which are designed to accomplish therapeutic or convenience objectives not offered by conventional or immediate-release forms (see: Bogner, R. H. Bioavailability and bioequivalence of extended-release oral dosage forms. U. S. Pharmacist 1997;22(Suppl.):3-12; see also: Scale-up of oral extended-release drug delivery systems: part I, an overview. Pharmaceutical Manufacturing 1985;2:23-27).

Extended-release dosage form: The United States Food and Drug Administration (F. D. A.) defines an extended-release dosage form as one that allows a reduction in dosing frequency to that presented by a conventional dosage form, e.g. a solution or an immediate-release dosage form (see, Bogner, R. H. Bioavailability and bioequivalence of extended-release oral dosage forms. US Pharmacist 1997;22(Suppl.):3-12; see also, Guidance for industry. Extended release oral dosage forms: development, evaluation, and application of the in vitro/in vivo correlations. Rockville, MD: Center for Drug Evaluation and Research, Food and Drug Administration, 1997).

Repeat action dosage form: These forms usually contain two single doses of medication, one for immediate release and the second for delayed release. Bi-layered tablets, for example, may be prepared with one layer of drug for immediate release with the second layer deigned to release drug later as either a second dose or in an extended-release manner.

Targeted-release dosage form: Targeted-release describes drug release directed towards isolating or concentrating a drug in a body region, tissue, or site for absorption or for drug action.

Envisaged within the scope of the present invention are MR dosage forms for at least oral administration, transdermal delivery, topical application, suppository delivery, transmucosal delivery, subcutaneous administration, subdermal administration, intramuscular administration, depot administration, implantable infusion, devices (both active and passive), delivery via bolus, parenteral administration and inhalation or insufflation.

Within the scope of the terms "modified" (ie; MR), "delayed" (ie; DR), "slow" or "sustained" (i.e. "SR"), sustained action (ie; SA), "prolonged" (ie; OA), "timed" (ie; TR), "long-acting" (ie; LA), "controlled" (i.e."CR"), and/or "extended" (i.e. "ER") release dosage units as used herein are any appropriate delivery form.

One example is oral delivery forms of tablet, capsule, lozenge, or the like form, or any liquid form such as syrups, aqueous solutions, emulsion and the like, capable of providing over the period of time between dosages an ongoing release of an effective level of the active ingredient.

Examples of dosage units for transdermal delivery include transdermal patches, transdermal bandages, and the like.

Included within the topical dosage forms are any lotion, stick, spray, ointment, paste, cream, gel, etc. whether applied directly to the skin or via an intermediary such as a pad, patch or the like but which again has a slow release action in delivery of the active agent into the body of the patient.

Examples of dosage units for suppository delivery include any solid dosage form inserted into a bodily orifice particularly those inserted rectally, vaginally and urethrally.

Examples of dosage units for transmucosal delivery include depositories solutions for enemas, pessaries, tampons, creams, gels, pastes, foams, nebulised solutions, powders and similar formulations containing in addition to the active ingredients such carriers as are known in the art to be appropriate.

Examples of dosage units for depot administration include pellets or small cylinders of active agent or solid forms wherein the active agent is entrapped in a matrix of biodegradable polymers, microemulsions, liposomes or is microencapsulated.

Examples of implantable infusion devices include any solid form in which the active agent is encapsulated within or dispersed throughout a biodegradable polymer or synthetic, polymer such as silicone, silicone rubber, silastic or similar polymer.

Alternatively dosage forms for infusion devices may employ liposome delivery systems.

Examples of dosage units for delivery via bolus include single or multiple administrations by intravenous injection, subcutaneous, subdermal, and intramuscular administration or oral administration.

Examples of dosage units for inhalation or insuffaltion include compositions comprising solutions and/or suspensions in pharmaceutically acceptable, aqueous, or organic solvents, or mixture thereof and/or powders.

STATEMENTS OF INVENTION

Accordingly in one aspect the present invention consists in a **method** of

(A) reversing in a mammal any cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences), and/or coronary artery structure damage (and its consequences),

(B) improving in a mammal by reversal of any damage any one or more systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (eg; as determined clinically, by ultrasound, MRI or other imaging),

(C) treating in a mammal by reversal of any damage any one or more of disorders of the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and the consequences thereof), and/or

(D) treating in a mammal by reversal of at least some of any damage arising from diabetic kidney disease, diabetic nephropathy and/or copper accumulation in the kidney and/or treating in a mammal by reversal of at least some of any damage to the renal arteries.

said method comprising or including

(i) diagnosing the mammal as being at risk and at least likely to be subject to some damage capable of being reversed, and

(ii) subjecting the mammal to chelation of copper values of the mammal capable of diminishment with a trientine moiety.

As used herein "trientine moiety" includes any administrable form of trientine (such as for example, the base form appropriately deliverably, salt forms such as the dihydrochloride, etc.).

Preferably the subjecting to chelation is with a regimen and/or dosage form(s) capable of providing a less pulsile exposure to trientine than has hitherto been the case with "qid" Wilson's Disease regimens.

Accordingly in another aspect the present invention in one aspect consists in a **method** of reversing in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient")

cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences) and/or

coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences),

which method comprises or includes the step of administration and/or self administration to the patient a slow or sustained release dosage form sufficient to provide effective chelation of copper for an overall diminishment thereof in the patient, said dosage form having as the or an active agent trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) ("trientine" including analogues thereof and/or prodrugs thereof).

Preferably the patient has been identified prior to treatment as being at risk.

As used herein "at risk" refers to mammals subjected to a risk assessment of a kind exemplified in the Journal of American Medical Association, May 16, 2001, Volume 285 No. 19, 2486-2497 where Framingham risk scoring which takes account of age, total cholesterol, HDL cholesterol, systolic blood pressure, treatment for hypertension and cigarette smoking is mentioned and to which can be added glucose abnormalities of any of the kinds herein described.

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

Accordingly in another aspect the present invention in one aspect consists in a **method** of improving by reversal of damage in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imaging),

which method comprises or includes the step of administration and/or self administration to the patient a slow or sustained release dosage form sufficient to provide effective chelation of copper for an overall diminishment thereof in the patient, said dosage form having as the or an active agent trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) ("trientine" including analogues thereof and/or prodrugs thereof).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

Accordingly in another aspect the present invention in one aspect consists in a **method** reversing in a patient at risk (I) being a diabetic human being or other diabetic mammal or (II) being a human being or other mammal with copper levels capable of diminishment

disorders of the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and consequences thereof),

which method comprises or includes the step of administration and/or self administration to the patient a slow or sustained release dosage form sufficient to provide effective chelation of copper for an overall diminishment thereof in the patient, said dosage form having as the or an active agent trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) ("trientine" including analogues thereof and/or prodrugs thereof).

Included within the categories of disease of patients that might usefully be targeted by the procedures of the present invention are any one or more of the following non exhaustive list:

diabetic cardiomyopathy,

diabetic acute coronary syndrome (eg; myocardial infarction – MI),

diabetic hypertensive cardiomyopathy,

acute coronary syndrome associated with impaired glucose tolerance (IGT),

acute coronary syndrome associated with impaired fasting glucose (IFG),

hypertensive cardiomyopathy associated with IGT, hypertensive cardiomyopathy associated with IFG,

ischaemic cardiomyopathy associated with IGT, ischaemic cardiomyopathy associated with IFG,

ischaemic cardiomyopathy associated with coronary heart disease (CHD),

acute coronary syndrome not associated with any abnormality of the glucose metabolism,

hypertensive cardiomyopathy not associated with any abnormality of the glucose metabolism,

ischaemic cardiomyopathy not associated with any abnormality of the glucose metabolism (irrespective of whether or not such ischaemic cardiomyopathy is associated with coronary heart disease or not), and

any one or more disease of the vascular tree including, by way of example, disease states of the aorta, carotid, cerebrovascular, coronary, renal, retinal, vasa nervorum, iliac, femoral, popliteal, arteriolar tree and capillary bed.

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

Preferably the effective amount is of trientine dihydrochloride.

Preferably the dosage of trientine dihydrochloride in sustained release is such that there is always less of the active ingredient in a patient's body than results from the 250 mg plus oral dosage forms for Wilson's Disease.

We recommend a sustained release dosage form or forms of trientine dihydrochloride preferably suitable for once daily administration and that provides sustained or controlled and long-lasting in vivo release. The form preferably delivers not more than 10% trientine hydrochloride in about 5 hours at an acid pH of about <4.5 and delivers greater than 50% of trientine hydrochloride in 12 hrs at a pH of about <6.5 in a controlled manner during in vivo and in vitro dissolution.

In a further aspect the present invention consists in **the use** of trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) (the "active agent(s)"), together with other material(s) appropriate for the dosage form, **in the manufacture of a sustained release dosage form** useful for reversing in a human being or other mammal at risk (preferably diabetic and/or with a copper level capable of diminishment) ("the patient")

cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences), and/or

coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **the use** of trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) (the "active agent(s)"), together with other material(s) appropriate for the dosage form, **in the manufacture of a sustained release dosage form** useful for improving by reversal of damage in a human being or other mammal at risk (preferably diabetic and/or with a copper level capable of diminishment) ("the patient") any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imaging).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **the use** of trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) (the "active

agent(s)") together with other material(s) appropriate for the dosage form, **in the manufacture** of a sustained release dosage form useful for improving by reversal of damage in a human being or other mammal at risk (preferably diabetic and/or with a copper level capable of diminishment) ("the patient") any one or more of at least some of any damage arising from diabetic kidney disease, diabetic nephropathy and/or copper accumulation in the kidney and/or at least some of any damage to the renal arteries

In a further aspect the present invention consists in **a transdermal patch, pad, wrap or bandage** ("patch") capable of being adhered or otherwise associated with the skin of a patient, said patch being capable of delivering an effective amount of trientine hydrochloride and/or its metabolites when so applied to a human being or other mammal at risk sufficient to reverse cardiac structure damage (and its consequences) selected from one or more of atrophy, loss of myocytes, expansion of the extra cellular space and increased deposition of extracelluar matrix and/or coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **a transdermal patch, pad, wrap or bandage** ("patch") capable of being adhered or otherwise associated with the skin of a patient, said patch being capable of delivering an effective amount of trientine, trientine hydrochloride and/or its metabolites when so applied to a human being or other mammal (preferably diabetic or predisposed thereto) sufficient to improve any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imaging).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **the use** of trientine, at least one salt of trientine and/or at least one metabolite of trientine and/or its salt(s) (the "active agent(s)", together with other material(s) appropriate for the dosage form, **in the manufacture of a sustained release dosage form** useful for ameliorating and/or reversing in a human being or other mammal (preferably diabetic and/or with a copper level capable of diminishment) ("the patient") disorders of the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and consequences thereof).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **a transdermal patch, pad, wrap or bandage** ("patch") capable of being adhered or otherwise associated with the skin of a patient, said patch being capable of delivering an effective amount of trientine hydrochloride and/or its metabolites when so applied to a human being or other mammal (preferably diabetic or predisposed thereto) sufficient to ameliorate and/or reverse disorders of the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and consequences thereof).

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In a further aspect the present invention consists in **a transdermal patch, pad, wrap or bandage** ("patch") capable of being adhered to or otherwise associated with the skin of a patient, said patch being capable of delivering an effective amount of trientine hydrochloride and/or its metabolites when so applied to a human being or other mammal sufficient to ameliorate and/or reverse at least some of any damage arising from diabetic kidney disease, diabetic nephropathy and/or copper accumulation in the kidney and/or to reverse at least some of any damage to the renal arteries.

In a another aspect the present invention consists in **an article of manufacturing** comprising:

a vessel containing as a CR, SR and/or ER dosage form or containing in CR, SR and/or ER dosage forms a pharmaceutically acceptable trientine moiety; and instructions for use of the trientine moiety for

(A) ameliorating and/or reversing in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences) and/or coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences),

(B) improving in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imagining,

(C) ameliorating and/or reversing in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") disorders or the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and the consequences thereof), and/or

(D) treating in a mammal by reversal of at least some of any damage arising from diabetic kidney disease, diabetic nephropathy and/or copper accumulation in the kidney and/or treating in a mammal, by reversal of at least some of any damage to the renal arteries.

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In another aspect the present invention consists in **an article of manufacture** comprising;

packaging material; and

contained within the packaging material a pharmaceutically acceptable trientine moiety in a CR, SR and/or ER dosage form,

wherein the packaging material has a label that indicates that the dosage form can be used for

(A) ameliorating and/or reversing in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") cardiac structure damage selected from one or more of atrophy, loss of myocytes, expansion of the extracellular space and increased deposition of extracellular matrix (and its consequences) and/or coronary artery structure damage selected from at least media damage (the muscle layer) and intima damage (the endothelial layer) (and its consequences),

(B) improving in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") any one or more of systolic function, diastolic function, contractility, recoil characteristics and ejection fraction (e.g. as determined clinically, by ultrasound, MRI or other imagining,

(C) ameliorating and/or reversing in (I) a diabetic human being or other diabetic mammal or (II) a human being or other mammal with copper levels capable of diminishment ("the patient") disorders or the heart muscle, macrovascular disease, microvascular disease and plaque rupture of athereomatous lesions of major blood vessels (and the consequences thereof), and/or

(D) treating in a mammal by reversal of any damage arising from diabetic kidney disease, diabetic nephropathy and copper accumulation in the kidney and/or treating in a mammal by reversal of damage to the renal arteries.

Preferably the dosage unit and/or dosage regimen is such as to provide an effective daily dosage to the patient of the trientine moiety (when expressed as the dihydrochloride salt of trientine, irrespective of whether or not the dosage unit includes that salt) of 4 g per day or below.

Preferably the dosage is from 1 mg to 4 g per day if given orally.

A preferred oral dose delivery (cumulative or otherwise) is in the range of from 200 mg to 4 g per day if given orally.

Preferably the daily dosage is such as to deliver 1.2 g per day or below.

In other aspects the dosage delivery is to provide, when expressed as trientine dihydrochloride, a delivery into the patient (irrespective of the dosage included in the

dosage unit or units) being administered of from 1 mg to 1.2 g per day. If orally administered the dosage is from 200 mg to 1.2 g per day.

Preferably the dosage is such as to deliver the trientine moiety in a dosage unit that administers the trientine moiety at a pH of from 7.2 to 7.6 (preferably a pH of 7.4± 0.1).

In another aspect the present invention consists in a method of administering an effective amount of Trientine formulated in a delayed release preparation (DR), a Slow Release preparation (SR), an Extended Release preparation (ER), a Controlled Release preparation (CR) and/or in a Repeat Action preparation (RA).

Preferably said formulations of DR, SR, ER, RA, or CR is suitable for use in the treatment of any of heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, Wilson's disease, or any form of cancer.

Preferably said formulations of DR, SR, ER, RA, or CR contains an effective dosage unit to the patient of the trientine from 1 mg to 600mg per unit.

Peferably the total daily dose rate is from between 5gms to 1mg.

Preferably the dosage unit will maintain a constant blood plasma concentration from between 1 hour to 24 hour.

In another aspect of the present invention consists in a formulation of trientine that maintains constant plasma concentrations of the drug for extended periods and is effective in removing copper from the body of patients with any of heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, Wilson's disease, or any form of cancer.

In another aspect of the present invention consists in a device containing trientine in a monolithic matrix device and employed for the treatment of any of heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, Wilson's disease, or any form of cancer.

Preferably said monolithic matrix device contains said trientine in a dispersed soluble matrix, in which said trientine becomes increasingly available as the matrix dissolves or swells.

Preferably said monolithic matrix device, includes but is not limited to one or more of the following excipients:

hydroxypropylcellulose (BP) or hydroxypropyl cellulose (USP); hydroxypropyl methylcellulose (BP, USP); methylcellulose (BP, USP); calcium carboxymethylcellulose (BP, USP); acrylic acid polymer or carboxy polymethylene (Carbopol) or Carbomer (BP, USP); or linear glycuronan polymers such as alginic acid (BP, USP), for example those formulated into microparticles from alginic acid (alginate)-gelatin hydrocolloid coacervate systems, or those in which liposomes have been encapsulated by coatings of alginic acid with poly-L-lysine membranes.

Alternatively, said monolithic matrix includes trientine particles dissolved in an insoluble matrix, from which trientine becomes available as an aqueous solvent enters the matrix through micro-channels and dissolves the trientine particles.

In another aspect the monolithic matrix contains said trientine particles in a lipid matrix or insoluble polymer matrix, including but not limited to preparations formed from Carnauba wax (BP; USP); medium-chain triglyceride such as fractionated coconut oil (BP) or triglycerida saturata media (PhEur); or cellulose ethyl ether or ethylcellulose (BP, USP).

Preferably said lipids are present in said monolithic matrix from between 20-40% hydrophobic solids w/w.

Preferably said lipids remain intact during the release process.

Preferably the device contains in addition to the trientine the following, but is not limited to; a channeling agent, such as sodium chloride or sugars, which leaches from the formulation, forming aqueous micro-channels (capillaries) through which solvent enters, and through which drug is released.

Alternatively the device is any hydrophilic polymer matrix, in which the trientine is compressed as a mixture with any water-swellable hydrophilic polymer.

Preferably the trientine contained in said hydrophilic polymer matrix is between 20 – 80% (w/w).

Preferably said hydrophilic polymer matrix contains in addition to the active agent any one or more of the following, a gel modifier such as one or more of a sugar, counter ions, a pH buffer, a surfactant, a lubricant such as a magnesium stearate and/or a glidant such as colloidal silicon dioxide.

In another aspect the present invention consists in any device containing an effective amount of the active agent comprising or including a rate-controlling membrane surrounding a drug reservoir and containing lactulose mixed with microcrystalline cellulose.

Preferably the ratio of lactulose to microcrystalline cellulose is 60:40.

We have determined in our trials referred to hereinafter that a divided dose of 1.2 g/day is effective for and yet (insofar as an instantaneous body level is concerned) in excess of dosage levels to be required chronically in practice for the purpose of amelioration and/or reversal of cardiac structure damage and/or coronary artery structure damage. Such a dose rate of 1.2 g per day is capable of being provided by the use of capsules of 300 mg trientine hydrochloride given half an hour before meals two being given in the morning and two being given at night.

Reference herein to "elevated" in relation to the presence of copper values will include humans having at least 10 mcg free copper/dL of serum when measured as discussed by Merck & Co Inc below.

A measurement of free copper [which equals total plasma copper minus ceruloplasmin-bound copper] can be made using the procedure disclosed in the Merck & Co Inc datasheet (www.Merck.com) for SYPRINE® (trientine dihydrochloride) capsules where they state in respect of the use of trientine dihydrochloride for the copper values excesses of Wilson's Disease:

"The most reliable index for monitoring treatment is the determination of free cooper in the serum, which equals the difference between quantitatively determined total copper and ceruloplasmin-copper. Adequately treated patients will usually have less than 10 mcg free copper/dL of serum.

Therapy may be monitored with a 24 hour urinary copper analysis periodically (i.e. every 6-12 months). Urine must be collected in copper-free glassware. Since a low copper diet should keep copper absorption down to less than one milligram a day, the patient probably will be in the desired state of negative copper balance if 0.5 to 1.0 milligram of copper is present in a 24-hour collection of urine".

The SYPRINE® data sheet in respect of the fast release forms in respect of Wilson's Disease says:

The data sheet for SYPRINE® reads systemic evaluation of dose and/or interval between dose has not been done. However, on limited clinical experience, the recommended initial dose of SYPRINE is 500-700 mg per day for pediatric patients and 750-1250 mg per day for adults given in divided doses two, three of four times daily. This may be increased to a maximum of 2000 mg per day for adults or 1500 mg per day for pediatric patients age 112 or under. The daily dose of SYPRINE should be increased only when the clinical response is not adequate or the concentration of free serum copper is persistently above 20 mcg/dl.

BRIEF DESCRIPTION OF THE DRAWINGS

We have conducted studies reliant on trientine dihydrochloride in the STZ rat model as well in humans and wish to describe the invention further by reference to the accompanying drawings in which:

Figure 1 (**Figure 1A** in colour and **Figure 1B** (the same as Figure 1A) in black and white) shows for the STZ rat model, animals made diabetic for more than six weeks,

having damaged cardiac structure with atrophy and loss of myocytes, expansion of the extracellular space, and increased deposition of extracellular matrix, these differences are observed between showing the non diabetic STZ rat cardiac tissue, the untreated diabetic rat tissue and the trientine dihydrochloride treated diabetic rat tissue,

Figure 2A shows in STZ diabetic rats compared with non diabetic rats cardiac function impairment that is largely corrected by chronic oral therapy with trientine dihydrochloride,

Figure 2B shows functional survival of working hearts arising from the isolated working heart model to form the basis of the data generation for Figure 2A,

Figure 3 shows how doses of trientine hydrochloride modifies copper excretion in the urine,

Figure 4 shows the absolute weight change with time of the period of the experiment,

Figure 5 is a diagram showing the bodyweight of the animals changing over the time period of the experiment,

Figure 6 shows the glucose levels of the animals changing over the time period of the experiment,

Figure 7 is a diagram showing cardiac output at various filling pressures,

Figure 8 is a diagram showing the coronary flow at various filling pressures,

Figure 8A is a diagram showing the coronary flow of Figure 8 normalised to final cardiac weight,

Figure 9 is a diagram showing the aortic flow with increasing preload,

Figure 10 is a diagram showing the peak pressure developed in the left ventricle at various filling pressures,

Figure 11 is a diagram showing the maximal rate of positive change in pressure development in the ventricle in response to increasing preload,

Figure 12 is a diagram showing the corresponding maximal rate of decrease in ventricular pressure in response to increasing preload,

Figure 13 shows the maximal rate of increase in pressure in the aortic outflow,

Figure 14 shows the corresponding maximal rate of decrease in the aortic pressure in response to increasing preload,

Figure 15 shows the percentage of functionally surviving hearts at each afterload pressure,

Figure 16 shows the cardiac output in response to increasing afterload,

Figure 17A shows the absolute change in coronary flow in response to increasing afterload,

Figure 17B shows the increases in coronary flow in response to increasing afterload normalized to heart weight,

Figure 18 shows the peak pressure developed in the left ventricle in response to increasing afterload,

Figure 19 shows the maximum rate of positive change in pressure development in the ventricle in response to increasing afterload,

Figure 20 shows the corresponding maximum rate of decrease in ventricular pressure in response to increasing afterload,

Figure 21 shows the maximum rate of positive change in pressure within the aortic outflow in response to increasing afterload,

Figure 22 shows the corresponding maximal rate of decrease in aortic pressure in response to increasing afterload,

Figure 23A shows diagrammatically how the extracted heart was attached to the modified apparatus,

Figure 23B shows in more detail the extracted heart as it was attached to the modified apparatus,

Figure 24 shows the urine excretion in diabetic and non diabetic animals in response to increasing doses of trientine,

Figure 25 shows volume of or an equivalent volume of saline (control) urine excreted in non diabetic and diabetic animals receiving increasing doses of trientine or an equivalent volume of saline,

Figure 26 shows copper excretion in the urine of diabetic and non diabetic animals receiving increasing doses of trientine or an equivalent volume of saline,

Figure 27 shows the same information in Figure 26 as urinary copper excretion per gram of bodyweight,

Figure 28 shows the total amount of copper excreted in non diabetic and diabetic animals administered saline or trientine,

Figure 29 shows the total amount of copper excreted per gram of bodyweight in animals receiving trientine or saline,

Figure 30 shows the iron excretion in urine of diabetic and non diabetic animals receiving increasing doses of trientine or an equivalent volume of saline,

Figure 31 shows the urinary iron excretion per gram of bodyweight in diabetic and non diabetic animals receiving trientine or saline,

Figure 32 shows the total urinary iron excretion in non diabetic and diabetic animals administered saline,

Figure 33 shows the total urinary iron excretion per gram of bodyweight in animals receiving trientine or saline,

Figure 34 shows the percentage of surviving hearts at each afterload pressure,

Figure 35 is a table comparing the copper and iron excretion in the animals receiving trientine or saline, which is a statistical analysis using a mixed linear model,

Figure 36 is a plasma concentration-time profiles of trientine after oral administration to four male patients, and

Figure 37 is a plasma concentration-time profiles of trientine after oral administration to four female patients..

DETAILED DESCRIPTION

In the STZ rat model, animals made diabetic for more than 6 weeks show damaged cardiac structure with atrophy and loss of myocytes, expansion of the extracellular space, and increased deposition of extracellular matrix. These appearances are both ameliorated and reversed by chronic oral therapy with trientine dihydrochloride. See, Figures 1A & 1B.

Using the isolated working heart model, cardiac function is severely impaired in STZ diabetic rats compared with non-diabetic animals. Cardiac dysfunction is largely

corrected by chronic oral therapy with trientine hydrochloride. See, Figures 2A & 2B. This is more clearly set out in the experimentation studies below.

EXPERIMENTS CONDUCTED

INTRODUCTION

Increased tissue copper has been implicated in mechanisms leading to diabetic nerve damage. We therefore hypothesized that tissue accumulation of trace metals might play a role in the mechanisms of diabetic damage in other tissues. Evidence from our earlier studies showed that 6 months of treatment with trientine appears to protect the hearts of diabetic Wistar rats from development of diabetic damage (cardiomyopathy) as judged by histology. In this study we investigated the doses of trientine required for copper and iron to be excreted in the urine, and also any possible difference between the excretion of these metals in diabetic and nondiabetic animals.

AIM

1. To compare the excretion profiles of copper and iron in the urine of normal and diabetic rats after acute intravenous administration of varying doses of trientine.
2. To ascertain that the acute intravenous administration of trientine has no acute adverse cardiovascular side effects.

METHODS

All methods used in this study were approved by the University of Auckland Animal Ethics Committee and were in accordance with The Animals Protection Act and Regulations of New Zealand.

Induction of diabetes.

Male Wistar rats (n = 28, 303 ± 2.9 g) were divided randomly into diabetic and nondiabetic groups. Following induction of anesthesia (5% halothane and 2l.min^{-1} O_2), animals in the diabetic group received a single intravenous dose of streptozotocin (STZ, 55 mg.kg^{-1} body weight, Sigma; St. Louis, MO) in 0.5 ml saline administered via the tail

vein. Nondiabetic animals received an equivalent volume of saline. Following injection, both diabetic and nondiabetic rats were housed in like-pairs and provided with access to normal rat chow (Diet 86 pellets; New Zealand Stock Feeds, Auckland, NZ) and deionized water ad libitum. Blood glucose and body weight were measure at day 3 following STZ/saline injection and then weekly throughout the study. Diabetes was identified by polydipsia, polyuria and hyperglycemia (> 11 $mmol.l^{-1}$, Advantage II, Roche Diagnostics, NZ Ltd).

Experimental procedure.

Six to seven weeks (mean = 44 ± 1 days) after administration of STZ, animals underwent either a control or drug experimental protocol. All animals were fasted overnight prior to surgery but continued to have ad libitum access to deionized water. Induction and maintenance of surgical anesthesia was by 3 - 5% halothane and 2l min^{-1} O_2. The femoral artery and vein were cannulated with a solid-state blood pressure transducer (Mikrotip™ 1.4F, Millar Instruments, Texas, USA) and a saline filled PE 50 catheter respectively. The ureters were exposed via a midline abdominal incision, cannulated using polyethylene catheters (external diameter 0.9 mm, internal diameter 0.5 mm) and the wound sutured closed. The trachea was cannulated and the animal ventilated at 70-80 $breaths.min^{-1}$ with air supplemented with O_2 (Pressure Controlled Ventilator, Kent Scientific, Connecticut, USA). The respiratory rate and end-tidal pressure (10-15 cmH_2O) were adjusted to maintain end-tidal CO_2 at 35-40 mmHg (SC-300 CO_2 Monitor, Pryon Corporation, Wisconsin, USA). Body temperature was maintained at 37°C throughout surgery and the experiment by a heating pad. Estimated fluid loss was replaced with intravenous administration of 154 $mmol.l^{-1}$ NaCl solution at a rate of 5 $ml.kg^{-1}.h^{-1}$.

Following surgery and a 20 min stabilization period, the experimental protocol was started. Trientine was administered intravenously over 60 s in hourly doses of increasing strength (0.1, 1.0, 10 and 100 $mg.kg^{-1}$ in 75 µl saline followed by 125 µl saline flush). Control animals received an equivalent volume of saline. Urine was collected in 15 min aliquots throughout the experiment in pre-weighed polyethylene eppendorf tubes. At the

end of the experiment a terminal blood sample was taken by cardiac puncture and the separated serum stored at -80°C until future analysis. Hearts were removed through a rapid mid-sternal thoracotomy and processed as described below.

Data acquisition.

Mean arterial pressure (MAP), heart rate (HR, derived from the MAP waveform) oxygen saturation (Nonin 8600V Pulse Oximeter, Nonin Medical Inc., Minnesota, USA) and core body temperature, were all continuously monitored throughout the experiment using a PowerLab/16s data acquisition module (AD Instruments, Australia). Calibrated signals were displayed on screen and saved to disc as 2 s averages of each variable.

Urine and tissue analysis.

Instrumentation: A Perkin Elmer (PE) Model 3100 Atomic Absorption Spectrophotometer equipped with a PE HGA-600 Graphite Furnace and PE AS-60 Furnace Autosampler was used for Cu and Fe determinations in urine. Deuterium background correction was employed. A Cu or Fe hollow-cathode lamp (Perkin Elmer Corporation) was used and operated at either 10 W (Cu) or 15 W (Fe). The 324.8 nm atomic line was used for Cu and the 248.3 nm atomic line for Fe. The slit width for both Cu and Fe was 0.7 nm. Pyrolytically coated graphite tubes were used for all analyses. The injection volume was 20 μL. A typical graphite furnace temperature program is shown below (table 1).

Table 1: GF-AAS temperature program

Procedure	*Temp / °C*	*Ramp / s*	*Hold / s*	*Int. Flow / mL min^{-1}*
Drying	90	1	5	300
	120	60	5	300
Pre-treatment	1250*	20	10	300
	20	1	10	300
Atomization – Cu / Fe	2300 / 2500	1	8	0
Post-treatment	2600	1	5	300

- A pre-treatment temperature of 1050 °C was used for tissue digest analyses

Cu, Fe and Zn in tissue digests were also determined at Hill Laboratories (Hamilton, New Zealand) using either a PE Sciex Elan-6000 or PE Sciex Elan-6100 DRC ICP-MS. The operating parameters are summarised in the table below (table 2).

Table 2 : Instrumental operating parameters for ICP-MS

Parameter	Value
Inductively coupled plasma	
Radiofrequency power	1500 W
Argon plasma gas flow rate	15 l.min^{-1}
Argon auxiliary gas flow rate	1.2 l.min^{-1}
Argon nebuliser gas flow rate	0.89 l.min^{-1}
Interface	
Sampler cone and orifice diameter	Ni / 1.1 mm
Skimmer cone and orifice diameter	Ni / 0.9 mm
Data acquisition parameters	
Scanning mode	Peak hopping
Dwell time	30 ms (Cu, Zn) / 100 ms (Fe)
Sweeps / replicate	20
Replicates	3
Sample uptake rate	1 ml.min^{-1}

Reagents: All reagents used were of the highest purity available and at least of analytical grade. GF-AAS standard working solutions of Cu and Fe were prepared by stepwise dilution of 1000 $mg.l^{-1}$ (Spectrosol standard solutions; BDH). Water was purified by a Millipore Milli-Q ultra-pure water system to a resistivity of 18 MΩ. Standard Reference Material 1577b Bovine Liver was obtained from the National Institute of Standards and Technology and used to evaluate the efficiency of tissue digestion. The results obtained are reported below (table 3).

Table 3 : GF-AAS and ICP-MS results for NIST SRM 1577b bovine liver*

Element	*Certified value*	*GF-AAS*	*ICP-MS*
Cu	160 ± 8	142 ± 12	164 ± 12
Fe	184 ± 15	182 ± 21	166 ± 14
Zn	127 ± 16	–	155 ± 42

* Measured in $\mu g.g^{-1}$ of dry matter.

Sample pretreatment:

Urine: Urine was collected in pre-weighed 1.5 ml micro test tubes (eppendorf). After reweighing, the urine specimens were centrifuged and the supernatant diluted 25:1 with 0.02 M 69 % Aristar grade HNO_3. The sample was stored at 4 °C prior to GF-AAS analysis. If it was necessary to store a sample for a period in excess of 2 weeks, it was frozen and kept at –20 °C.

Heart: Following removal from the animal, the heart was cleaned of excess tissue, rinsed in buffer to remove excess blood, blotted dry and a wet ventricular weight recorded. Using titanium instruments a segment of left ventricular muscle was dissected and placed in a pre-weighed 5.0 ml polystyrene tube. The sample was freeze-dried overnight to constant weight before 0.45 ml of 69% Aristar grade HNO_3 was added. The sample tube was heated in a water bath at 65 °C for 60 minutes. The sample was brought to 4.5 ml with Milli-Q H_2O. The resulting solution was diluted 2:1 in order to reduce the HNO_3

concentration below the maximum permitted for ICP-MS analysis. The remaining left ventricular tissue was stored in 10% formalin and later processed for transmission electron microscopic examination and histochemical analysis.

Serum: Terminal blood samples were centrifuged and serum treated and stored as per urine until analysis. From the trace metal content of serum from the terminal blood sample and urine collected over the final hour of the experiment, renal clearance was calculated using the following equation:

$$\text{renal clearance of trace metal} = \frac{\text{concentration of metal in urine } (\mu g.\ \mu l^{-1}) * \text{rate of urine flow } (\mu l.min^{-1})}{\text{concentration of metal in serum } (\mu g.\ \mu l^{-1})}$$

Statistical analysis.

All values are expressed as mean ± SEM and P values < 0.05 were considered statistically significant. Student's unpaired t-test was initially used to test for weight and glucose differences between the diabetic and control groups. For comparison of responses during drug exposure, statistical analyses were performed using analysis of variance (Statistica for Windows v.6.1, SAS Institute Inc., Calfornia, USA). Subsequent statistical analysis was performed using a mixed model repeated measures ANOVA design (see: Figure 35 and Table 7 *below*).

RESULTS

Table 4 : Blood glucose, body weight and food consumption in diabetic versus nondiabetic animals.

	Diabetic	**Nondiabetic**
Body weight prior to STZ/saline	303 ± 3 g	303 ± 3 g
Blood glucose 3 days following STZ/saline	*25 ± 2 mmol.l^{-1}	5 ± 0.2 mmol.l^{-1}
Daily food consumption	*58 ± 1 g	28 ± 1 g
Blood glucose on experimental day	*24 ± 1 mmol.l^{-1}	5 ± 0.2 mmol.l^{-1}
Body weight on experimental day	*264 ± 7 g	434 ± 9 g

Diabetic animals n = 14, nondiabetic animals n = 14. Values shown as mean ± SEM. Asterisk indicates a significant difference ($P < 0.05$).

Effects of STZ on blood glucose and body weight (Table 4)

Blood glucose increased to 25 ± 2 mmol.l^{-1} three days following STZ injection. Despite a greater daily food intake, diabetic animals lost weight whilst nondiabetic animals continued to gain weight during the 44 days following STZ/saline injection. On the day of the experiment blood glucose levels were 24 ± 1 and 5 ± 0 mmol.l^{-1} and body weight 264 ± 7 g and 434 ± 9 g for diabetic and nondiabetic animals respectively.

Cardiovascular variables during infusion

Baseline levels of MAP during the control period prior to infusion were not significantly different between nondiabetic and diabetic animals (99 ± 4 mmHg). HR was significantly lower in diabetic than nondiabetic animals (287 ± 11 and 364 ± 9 bpm respectively, $P < 0.001$). Infusion of trientine or saline had no effect on these variables except at the highest dose where MAP decreased by a maximum of 19 ± 4 mmHg for the 2 min following administration and returned to pre-dose levels within 10 min. Body temperature and oxygen saturation remained stable in all animals throughout the experiment.

Urine Excretion

Diabetic animals consistently excreted significantly more urine than nondiabetic animals except in response to the highest dose of drug (100 mg.kg^{-1}) or equivalent volume of saline (Fig. 24). Administration of the 100 mg.kg^{-1} dose of trientine also increased urine excretion in nondiabetic animals to greater than that of nondiabetic animals receiving the equivalent volume of saline (Fig. 25). This effect was not seen in diabetic animals.

Urinary excretion of Cu and Fe

Analysis of the dose response curves shows that, at all doses, diabetic and nondiabetic animals receiving drug excreted more Cu than animals receiving an equivalent volume of saline (Fig. 26). To provide some correction for the effects of lesser total body growth of the diabetic animals, and thus to allow more appropriate comparison between diabetic and nondiabetic animals, excretion rates of trace elements were also calculated per gram of body weight. Figure 27 shows that diabetic animals had significantly greater copper excretion per gram of body weight in response to each dose of drug than did nondiabetic animals. The same pattern was seen in response to saline, however the effect was not always significant.

Total copper excreted over the entire duration of the experiment was significantly increased in both nondiabetic and diabetic animals administered trientine compared with their respective saline controls (Fig. 28). Diabetic animals receiving drug also excreted more total copper per gram of body weight than nondiabetic animals receiving drug. A similar, but not significant trend was seen in response to saline administration (Fig. 29).

In comparison, iron excretion in both diabetic and nondiabetic animals receiving trientine was not greater than animals receiving an equivalent volume of saline (Fig. 30). Analysis per gram of body weight shows diabetic animals receiving saline excrete significantly more iron than nondiabetic animals, however this trend was not evident between diabetic and nondiabetic animals receiving trientine (Fig. 31). Total iron excretion in both diabetic and nondiabetic animals receiving drug was not different from animals receiving saline (Fig 32). In agreement with analysis of dose response curves, total iron excretion per gram of body weight was significantly greater in diabetic animals receiving saline than nondiabetic animals but this difference was not seen in response to trientine (Fig 33).

Serum content and renal clearance of Cu and Fe (Table 5)

While there was no significant difference in serum copper content, there was a significant increase in renal clearance of copper in diabetic animals receiving drug compared with diabetic animals receiving saline. The same pattern was seen in

nondiabetic animals, although the trend was not statistically significant ($P = 0.056$). There was no effect of drug or state (diabetic versus nondiabetic) on serum content or renal clearance of iron.

Table 5. Serum content and renal clearance of Cu and Fe in diabetic and nondiabetic animals receiving drug or saline.

	Diabetic		**Nondiabetic**	
	trientine $n = 6$	*Saline* $n = 7$	*trientine* $n = 4$	*Saline* $n = 7$
Serum Cu ($\mu g.\mu l^{-1} \times 10^{-4}$)	7.56 ± 0.06	9.07 ± 1.74	7.11 ± 0.41	7.56 ± 0.62
Serum Fe ($\mu g.\mu l^{-1} \times 10^{-4}$)	35.7 ± 7.98	63.2 ± 16.4	33.6 ± 1.62	31.4 ± 8.17
Renal clearance Cu ($\mu l.min^{-1}$)	*28.5 ± 4.8	1.66 ± 0.82	5.8 ± 0.28	19.9 ± 6.4
Renal clearance Fe ($\mu l.min^{-1}$)	0.25 ± 0.07	0.38 ± 0.15	0.46 ± 0.22	0.11 ± 0.03

Values shown as mean ± SEM. Asterisk indicates a significant difference ($P < 0.05$) between diabetic animals receiving trientine and diabetic animals receiving an equivalent volume of saline.

Metal content of cardiac tissue (Table 6)

Wet heart weights in diabetic animals were significantly less than those in nondiabetic animals while heart/body weight ratios were increased. In some animals cardiac tissue was also analysed for Cu and Fe content. There was no significant difference in content of either metal between diabetic and nondiabetic animals.

Table 6: Heart weight, heart weight/body weight ratios and trace metal content of heart tissue in diabetic versus nondiabetic animals.

	Diabetic	Nondiabetic
Wet heart weight	*0.78 ± 0.02 g	1.00 ± 0.02 g
Heart weight/body weight	*2.93 ± 0.05 $mg.g^{-1}$	2.30 ± 0.03 $mg.g^{-1}$
Cu content	26.7 ± 1.4 $\mu g.g^{-1}$ dry tissue	27.9 ± 0.7 $\mu g.g^{-1}$ dry tissue
Fe content	296 ± 15 $\mu g.g^{-1}$ dry tissue	299 ± 9 $\mu g.g^{-1}$ dry tissue

Diabetic animals: n = 5; nondiabetic animals: n = 10. Values shown as mean ± SEM. Asterisk indicates a significant difference ($P < 0.05$).

THE EFFICACY OF TRIENTINE TO RESTORE CARDIAC FUNCTION IN STZ DIABETIC RATS

INTRODUCTION

Increased tissue copper has been implicated in mechanisms leading to diabetic nerve damage. We therefore hypothesized that tissue accumulation of trace metals might play a role in the mechanisms of diabetic damage in other tissues. Evidence from our earlier studies showed that 6 months of treatment with trientine appears to protect the hearts of diabetic Wistar rats from development of cardiac damage (diabetic cardiomyopathy), as judged by histology. However, it was unknown whether this histological improvement translates into an improvement in cardiac function, a finding that would lend further support to use of trientine therapy in clinical applications.

AIM

The aim of this study was to use an isolated-working-heart model to compare cardiac function in trientine-treated STZ diabetic rats with that in untreated STZ diabetic rats and non-diabetic control rats.

METHOD

Animals

The animals used in these experiments received care that complied with the "Principles of Laboratory Animal Care" (National Society for Medical Research), and the study was approved by the University of Auckland Animal Ethics Committee.

Male albino Wistar rats weighing 330-430g were assigned to three experimental groups as follows (table 7):

Table 7: Experimental groups

Group	Code	N	Treatment
Group A	STZ	8	Diabetes for 13 weeks
Group B	STZ/D6	8	Diabetes for 13 weeks Drug therapy week 7-13
Group C	Sham	9	Non-diabetic controls

STZ = Streptozotocin
D6 = trientine treatment for 6 consecutive weeks

Diabetes was induced by intravenous streptozotocin (STZ; Sigma; St. Louis, MO). All rats were given a short inhalational anaesthetic (Induction: 5% halothane and 2 L/min oxygen, maintained on 2% halothane and 2 L/min oxygen). Those rats assigned to be in the two diabetic groups then received a single intravenous bolus dose of STZ (57 mg/kg body weight) in 0.5 ml of 0.1% saline administered via a tail vein. Non-diabetic sham-treated animals received an equivalent volume of 0.9% saline alone. Diabetic and non-diabetic rats were housed in like-pairs and provided with free access to normal rat chow (Diet 86 pellets; New Zealand Stock Feeds, Auckland, NZ) and deionized water *ad libitum*. Animals were housed at 21 degrees and 60% humidity in standard rat cages with a sawdust floor that was changed daily.

Blood glucose was measured in tail-tip capillary blood samples (Advantage II, Roche Diagnostics, NZ Ltd). Sampling was performed on all groups at the same time of the day. Blood glucose and body weight were measured on day 3 following STZ or saline injection and then weekly throughout the study. Diabetes was confirmed by presence of polydipsia, polyuria and hyperglycemia (>11 $mmol.L^{-1}$).

In the drug treated diabetic group, trientine was prepared in the drinking water for each cage to a concentration of 50 mg/L. Animals consumed about 260 ml water per day once diabetes was established, to yield a total drug dose per day of approximately 13 mg/L. The drug-containing drinking water was administered continuously from week 7 until the animal was sacrificed at week 13. At week 7 diabetic animals are known to have established cardiomyopathy, as shown by our preliminary studies and confirmed in the literature. See Rodrigues, B. Xiang McNeill JH. Of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. *Diabetes.* 37(10):1358-64, 1998 October.

On the last day of the experiment, animals were anesthetized (5% halothane and 2 $L.min^{-1}$ O_2), and heparin (500 $IU.kg^{-1}$) (Weddel Pharmaceutical Ltd., London) administered intravenously via tail vein. A 2 ml blood sample was then taken from the inferior vena cava and the heart was then rapidly excised and immersed in ice-cold Krebs-Henseleit bicarbonate buffer to arrest contractile activity. Hearts were then placed in the isolated perfused working heart apparatus.

Perfusion

The aortic root of the heart was immediately ligated to the aortic cannula. Retrograde (Langendorff) perfusion at a hydrostatic pressure of 100 cm H_2O and at 37°C was established and continued for 5 min while cannulation of the left atrium via the pulmonary vein was completed. The non-working (Langendorff) preparation was then converted to the working heart model by switching the supply of perfusate buffer from the aorta to the left atrium at a filling pressure of 10 cm H_2O. The left ventricle spontaneously ejected into the aortic cannula against a hydrostatic pressure (afterload) of 76 cmH_2O (55.9 mmHg). The perfusion solution was Krebs-Henseleit bicarbonate buffer (mM: KCl 4.7, $CaCl_2$ 2.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, NaCl 118, and $NaHCO_3$ 25), pH 7.4 containing 11 mM glucose and was continuously gassed with 95% O_2:5% CO_2. Buffer was continuously filtered in-line (initial 8, following 0.4 cmc cellulose acetate filters; Sartorius, Germany). The temperature of the entire perfusion apparatus was maintained

by water jackets and buffer temperature was continuously monitored and adjusted to maintain hearts at 37°C throughout perfusion.

Pressure monitoring and pacing

A modified 24g plastic cannula (Becton Dickson, Utah, USA) was inserted into the left ventricle via the apex of the heart using the normal introducer-needle. This was attached to a SP844 piezo-electric pressure transducer (AD Instruments) to continuously monitor left ventricular pressure. Aortic pressure was continuously monitored through the side arm of the aortic cannula with a pressure transducer (Statham Model P23XL, Gould Inc., CA, USA). The heart was paced (Digitimer Ltd, Heredfordshire, England) at a rate of 300 bpm by means of electrodes attached to the aortic and pulmonary vein cannulae using supra-threshold voltages with pulses of 5-ms duration from the square wave generator.

Aortic flow measurements

Aortic flow was recorded by an in-line flow meter (Transonic T206, Ithaca, NY, USA) and coronary flow was measured by timed 30 s collection of the coronary vein effluent at each time point step of the protocol.

Working heart apparatus

This working heart apparatus was a variant of that originally described by Neely JR. Liebermeister H. Battersby EJ. Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. American Journal of Physiology. 212(4):804-14, 1967 Apr. Neely JR. Liebermeister H. Battersby EJ. Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. American Journal of Physiology. 212(4):804-14, 1967 Apr. Neely JR. Liebermeister H. Battersby EJ. Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. American Journal of Physiology. 212(4):804-14, 1967 Apr.. Our modified apparatus allowed measurements of cardiac function at different preload pressures (Figure 23A and Figure 23B). This was achieved by constructing the apparatus so that the inflow height of

the buffer coming to the heart could be altered through a series of graduated steps in a reproducible manner. As in the case of the afterload, the outflow tubing from the aorta could be increased in height to provide a series of defined afterload pressures; these have been converted to mmHg for presentation in the results.

Data collection

All data from the pressure transducers and flow probe were collected (Powerlab 16s data acquisition machine; AD Instruments, Australia). The data processing functions of this device were used to calculate the first derivative of the two pressure waves (ventricular and aortic). The final cardiac function data reported comprised:

Cardiac output*; aortic flow; coronary flow; mean left ventricular pressure developed (MLVDP); maximum rate of ventricular pressure development (+dP/dt)**; maximum rate of ventricular pressure relaxation (-dP/dt)**; maximum rate of aortic pressure development (aortic +dP/dt); maximum rate of aortic relaxation (aortic -dP/dt).

[*Cardiac output (CO) is the amount of buffer pumped per unit time by the heart and is comprised of buffer that is pumped out of the aorta as well as the buffer pumped into the coronary vessels. This is an overall indicator or cardiac function.

** +dP/dt is the rate of change of ventricular (or aortic pressure) and correlates well with the strength of the contraction of the ventricle (contractility). It can be used to compare contractile abilities of different hearts when at the same preload (Textbook of Medical Physiology, Ed. A.Guyton. Saunders company 1986). -dP/dt is an accepted measurement of the rate of relaxation of the ventricle].

Protocol

The experiment was divided into two parts, the first with fixed afterload and variable preload the second, which immediately followed on from the first, with fixed preload and variable afterload.

I) Fixed Afterload and changing Preload

After the initial cannulation was completed, the heart was initially allowed to equilibrate for 6 min at 10 cm H_2O of water atrial filling pressure and 76 cm H_2O water

afterload. During this period the left ventricular pressure transducer cannula was inserted and the pacing unit started. Once the heart was stable, the atrial filling pressure was then reduced to 5 cm H_2O of water and then progressively increased in steps of 2.5 cm H_2O over a series of 7 steps to a maximum of 20 cm H_2O. The preload was kept at each filling pressure for 2 min, during which time the pressure trace could be observed to stabilize and the coronary flow was measured. On completion of the variable preload experiment, the variable afterload experiment was immediately commenced.

II) Fixed Preload and changing Afterload

During this part of the experiment the filling pressure (preload) was set at 10 cm H_2O and the afterload was then increased from 76 cm H_2O (55.9 mmHg) in steps of 8 cm H_2O (5.88 mmHg); again each step was of 2 min duration. The maximum height (afterload) to which each individual heart was ultimately exposed, was determined either by attainment of the maximal available afterload height of 145 cm H_2O (106.66 mmHg), or the height at which measured aortic flow became less than 5 ml/min. In the later situation, the heart was considered to have "functionally failed". To ensure that this failure was mechanical and not due to other causes (e.g. ischaemic damage) all hearts were then returned to the initial perfusion conditions (preload 10 cm H_2O; afterload 75 cm H2O) for 4 min to confirm that pump function could be restored.

At the end of this period the hearts were arrested with a retrograde infusion of 0.4 ml of cold KCL (24 mM). The atria and vascular remnants were then excised, the heart blotted dry, and the ventricles incised midway between the apex and atrioventricular sulcus. Measurements of the ventricular wall thickness were then made using a micro-caliper (Absolute Digimatic, Mitutoyo Corp, Japan).

ANALYSIS

Data from the Powerlab was extracted by averaging 1 min intervals from the stable part of the electronic trace generated from each step in the protocol. The results from each group were then combined and analysed for differences between the groups for the various cardiac function parameters (aortic flow, cardiac flow, MLVDP, LV or aortic +/- dP/dt).

RESULTS

Animals

The weights of the animals at the end of the experimental period are shown in Table 8. Because there was a small difference in the initial weights of the animals, a graph of the percentage change in weight for each group has been included (Figure 5B).

Table 8: Initial and Final Animal weights (mean ± SD)

	Number (n)	Treatment	Initial weight (g)		Final weight (g)	
Group A	9	STZ	361 ±12	*	221±27	*
Group B	8	STZ/D6	401 ± 33		290±56	
Group C	8	Sham	361 ±16	*	574±50	

* P < 0.01

Diabetic status

Blood glucose values for the three groups of rats are presented in Figure 6. Generally, the presence of diabetes was confirmed established within 3-5 days following the STZ injection. The Sham control group remained normoglycaemic throughout the experiment, and treatment with the drug made no difference to their blood glucose profile (p=ns), as expected.

Heart weights

Final heart weight and ventricular wall thickness measurements are presented in Table 9.

Table 9: Final heart weights (g) and per g of animal body Weight (BW) (mean ± SD)

Group	Heart weight (g)		Heart weight (g) /BW (g)		Left Ventricular wall thickness (mm)		Left Ventricular wall thickness (mm)/BW (g)	
Sham	1.58 ± 0.13§		0.0028±0.0002§		3.89±0.38§		0.0068±0.0009§	
STZ/D6	1.18 ± 0.24	ns	0.0041±0.0005	*	3.79±0.52	ns	0.0127±0.0027	ns
STZ	1.03 ± 0.17		0.0047±0.0004		3.31±0.39		0.0152±0.0026	

- P=0.02
- § = Highly significant when compared with the other 2 groups

Part I results

The following graphs of Figures 7 to 14 demonstrate cardiac performance parameters of the animals (STZ diabetic; STZ diabetic +drug; and sham-treated controls) while undergoing increasing atrial filling pressure (5-20 cmH_2O, preload) with a constant afterload of 75 cm H_2O.

Cardiac output (Figure 7) is the sum to the aortic flow (Figure 9) and the coronary flow as displayed in Figure 8. Since the control hearts and experimental groups have significantly different final weights, the coronary flow is presented (Figure 8A) as the flow normalized to heart weight [Note that coronary flow is generally proportional to cardiac muscle mass, and therefore to cardiac weight].

The mean left ventricular developed pressure (MLVDP) is summarized in Figure 10. The first derivative of the pressure curve gives the rate of change in pressure development in the ventricle with each cardiac cycle and the maximum positive rate of change (+dP/dt) value is plotted in Figure 11. The corresponding maximum rate of relaxation (-dP/dt) is in Figure 12. Similar information appears in Figure 13 and Figure 14, in which the maximum positive and negative rates of change in pressure within the aortic outflow tubing are shown.

All results are mean ± sem.

Sham (n=9), STZ diabetic (n=8), STZ Diabetic + Drug (D6) (n=8)

# $p<0.01$ STZ/D6 v STZ

*$p<0.01$ STZ/D6 v Sham

Part II results

A similar set of graphs are presented in Figures 15 to 22 and Figure 34 which demonstrate equivalent functional outcomes, but this time under conditions of constant preload and changing afterload. Results are mean ± sem.

Note: In part I of the experiment, all hearts remained functional throughout all the changes in preload. However in this section, the increased work associated with the higher afterloads was used as an additional indicator of cardiac function. The numbers of functionally surviving hearts (n) in each group varies at higher afterload levels. This

attrition reflects functional failure of the heart at the stated level of afterload. (See Table 10 and Figure 34).

Table 10: Cardiac survival at each afterload pressure

Number surviving						Percentage			
Afterload (mmHg)	STZ	STZ/D6	Sham			Afterload (mmHg)	STZ	STZ/D6	Sham
55.9	8	8	9			55.9	100%	100%	100%
61.8	8	8	9			61.8	100%	100%	100%
67.7	8	8	9			67.7	100%	100%	100%
71.4	6	8	9			71.4	75%	100%	100%
77.2	5	8	9			77.2	63%	100%	100%
83.1	4	8	9			83.1	50%	100%	100%
88.3	3	7	9			88.3	38%	88%	100%
94.9	1	6	9			94.9	13%	75%	100%
100.8	0	5	9			100.8	0%	63%	100%
106.7	0	1	9			106.7	0%	13%	100%

SUMMARY

- Treatment with trientine had no obvious effect on blood glucose concentrations in the two diabetic groups (as expected).
- There was a small but significant improvement in the cardiac weight /body weight ratio in the trientine-treated diabetic group compared to that of the untreated diabetic group.
- When the Preload was increased with the Afterload held constant, cardiac output was restored to Sham values. Both the aortic and absolute coronary flows improved in the drug treated group.
- When the coronary flow was normalized to heart weight, the drug treated group still showed improved flows at lower filling pressures than did the untreated diabetic animals.

- Indicators for ventricular contraction and relaxation were both significantly improved in the drug treated group compared to equivalent values in the untreated diabetic group. The improvement restored function to such an extent that there was no significant difference between the drug treated and the sham-treated control groups.
- The aortic transducer measures of pressure change also showed improved function in the drug treated group
- When afterload was increased in the presence of constant preload, it was observed that the heart's ability to function at higher afterload was greatly improved in the drug treated diabetic group compared to the untreated diabetic group. When 50% of the untreated hearts had failed,100% of the drug treated hearts were still functioning.
- Compared to the untreated diabetic hearts, the response of the drug treated diabetic hearts showed significant improvements in several variables: cardiac output, aortic flow, coronary flow, as well as improved ventricular contraction and relaxation indices in both the ventricular and aortic pressure wave profiles.

CONCLUSION

These preliminary data suggest that treatment of STZ diabetic rats with trientine dramatically improves several measures of cardiac function.

Additional relevant notes:

Other published studies in Wistar rats have shown that the functional changes of the cardiomyopathy of STZ rats is

- Cardiomyopathy is present after 5-6 weeks following STZ
  Rodrigues B, Am J Physiol 251 H571-H580 1986
- Not related to heart size: since small weight matched hearts have same function as larger control hearts (we confirmed this in our pilot study as well)
- Not due to malnutrition effects of low insulin: since calorie deprived rats with hearts of the same weight had the same function as larger control hearts

- Not substrate dependent: addition of extra insulin and increased glucose did not reverse the functional deficit
- Probably not due to the STZ itself: since animals given STZ in this study but that failed to go on to develop diabetes, did not get a change in heart function
  Penpargkul S et al, Cir Res 4: 911-921 1980

Conclusions:

Administration of oral Trientine for 6 weeks in Wistar rats with previously established diabetes of 7 weeks duration (a duration known from the literature and our first pilot study, to be associated with significant cardiomyopathy) resulted in a global improvement in cardiac function. This improvement was demonstrated by measures of improved contractile function (LVDP most clearly seen in the afterload experiment +dP/dT) and a reduction in ventricular stiffness (-dP/dT). These parameters improved in the presence of either increasing preload or after-load protocols.

SUMMARY

The acute effect of intravenous trientine administration on urinary excretion of copper and iron was studied in anesthetized, diabetic (6 weeks of diabetes, Streptozotocin induced) and nondiabetic rats. Animals were assigned to one of four groups: diabetic + trientine, diabetic + saline, nondiabetic + trientine, nondiabetic + saline. Drug, or an equivalent volume of saline, was administered hourly in doses of increasing strength (0.1, 1.0, 10, 100 $mg.kg^{-1}$) and urine was collected throughout the experiment in 15 min aliquots. A terminal blood sample was taken and cardiac tissue harvested.

Analysis of urine samples showed the following main points:

- At all drug doses, diabetic and nondiabetic animals receiving drug excreted more Cu (μg) than animals receiving an equivalent volume of saline.
- When analsyed per gram of bodyweight, diabetic animals excreted significantly more copper ($\mu g.gBW^{-1}$) at each dose of trientine than did nondiabetic animals. The same pattern was seen in response to saline but the effect was not significant at every dose.

- At most doses, in diabetic animals iron excretion (μg) was greater in animals administered saline than in those administered drug. In nondiabetic animals there was no difference between iron excretion in response to saline or trientine administration.
- Analysis per gram of body weight shows no difference between iron excretion in nondiabetic and diabetic animals receiving trientine. Diabetic animals receiving saline excrete more iron per gram of bodyweight than nondiabetic animals receiving saline.

Analysis of heart tissue showed no significant difference in content of either metal between diabetic and nondiabetic animals, nor any effect of drug on cardiac content of iron and copper.

Renal clearance calculations showed a significant increase in clearance of copper in diabetic animals receiving trientine compared with diabetic animals receiving saline. The same trend was seen in nondiabetic animals but the effect was not significant. There was no effect of trientine on renal clearance of iron.

In summary, trientine-treatment effectively increases copper excretion in both diabetic and nondiabetic animals. The excretion of copper in urine following trientine administration, is greater per gram of bodyweight in diabetic than in nondiabetic animals. Iron excretion was not increased by trientine treatment in either diabetic or nondiabetic animals.

Statistical methods

Data for each dose level were analysed using a mixed linear model (PROC MIXED; SAS, Version 8). The model included diabetes, drug and their interaction as fixed effects, time as a repeated measure, and rats as the subjects in the dataset. Complete independence is assumed across subjects. The full model was fitted to each dataset using a maximum likelihood estimation method (REML) fits mixed linear models (i.e., fixed and random effects models). A mixed model is a generalization of the standard linear model, the generalization being that you can analyse data generated from several sources of variation instead of just one. A level of significance of 0.05 was used for all tests.

RESULTS

Copper

Diabetic rats excreted significantly higher levels of copper across all dose levels. Baseline copper excretion was also significantly higher in diabetic rats compared to and prior to drug administration. The drug resulted in a significantly higher excretion of copper compared to saline at all does levels. There was no difference at baseline levels between the drug and saline groups. The interaction effect for the model was significant at dose levels of 1.0 $mg.kg^{-1}$ and above. The presence of a significant interaction term means that the influence of each effect varies with the level of the other effect. Therefore, the outcome of a significant interaction between the diabetes and drug factors in increased copper excretion above the predicted additive effects of these two factors.

Iron

Diabetic rats in the saline only group excreted significantly higher levels of iron at all dose levels. This resulted in all factors in the model being significant across all dose levels.

Statistical analysis using a mixed linear model.

Data for each dose level were analysed using a mixed linear model (PROC MIXED; SAS, Version 8). The model included *diabetes*, *drug* and their interaction as fixed effects, *time* as a repeated measure, and *rats* as the subjects in the dataset. Complete independence is assumed across subjects. The full model was fitted to each dataset using a maximum likelihood estimation method (REML) fits mixed linear models (i.e., fixed and random effects models). A mixed model is a generalization of the standard linear model, the generalization being that you can analyse data generated from several sources of variation instead of just one. A level of significance of 0.05 was used for all tests.

Results from application of the above mixed linear model to the experimental analysis (Figure 35).

Diabetic rats excreted significantly higher levels of copper across all dose levels. Baseline copper excretion was also significantly higher in diabetic rats compared to and

prior to drug administration. The drug resulted in a significantly higher excretion of copper compared to saline at all dose levels. There was no difference at baseline levels between the drug and saline groups.

The interaction effect for the model was significant at dose levels of 1.0 $mg.kg^{-1}$ and above. The presence of a significant interaction term means that the influence of one effect varies with the level of the other effect. Therefore, the outcome of a significant interaction between the diabetes and drug factors is increased copper excretion above the predicted additive effects of these two factors.

Diabetic rats in the saline only group excreted significantly higher levels of iron at all dose levels. This resulted in all factors in the model being significant across all dose levels.

HUMAN STUDIES – Phase II

Table 11 shows baseline information on 30 patients with long-standing type 2 diabetes, no clinical evidence of coronary artery disease and abnormal diastolic function who participated in a 6-month randomised, double blind, placebo controlled study of chronic oral therapy with trientine dihydrochloride.

Table 11: Characteristics of Study Participants

	Placebo	Trientine dihydrochloride
N	15	15
Median age (years)	54 (range 43-64)	52 (range 33-69)
% female	44%	56%
Median duration of diabetes (years)	10 (6-24)	8 (4-15)
Mean body mass index (kg/m^2)	32 (SD 5)	34 (SD 5)
% hypertensive	64%	80%
% HbA_{1c}>8	93%	80%

MRI scans of the heart at baseline and 6-months showed a significant reduction in LV mass and a significant improvement in diastolic function measured as a change in apical rotation (AR) at the end of systole. See, Table 12. These effects indicate improved structure and function in the human heart following 6 months of trientine therapy.

Table 12 : Phase II: INFO-Cardiac MRI Results

	Placebo (n=15)	GC811007 (n=15)	P
Baseline LVM	202.17	207.45	0.778
Δ LVM 1-6mo	+6.57	-10.49	0.0045
Baseline AR	12.37	12.49	0.931
Δ AR 1-6mo	+0.81	-2.19	0.029

Therefore, an equivalent dose of oral trientine dihydrochloride corrected for weight (15 mg/kg) is effective in both rats and humans.

Compared with non-diabetic animals, STZ diabetic rats show a 2-fold to 10-fold excess of urinary copper excretion following parenteral administration of trientine hydrochloride. Cupuresis is rapid with excess urinary copper excretion manifest within the first 15 minutes of an intravenous bolus injection. Trientine dihydrochloride is effective by parenteral injection in a 10-fold to 100-fold lower dose than the current oral therapy. See, Figure 3 and our patent application filed simultaneously herewith directed to parenteral dosage forms and their use.

In the preceeding section, animal data have been presented which show that administration of trientine dihydrochloride; (Merck Index 12th Ed., 9796) in a manner that maintains constant drug levels, has the property of effectively treating established heart failure in diabetic rats. The data clearly demonstrate that the drug can reverse heart failure while essentially causing the heart to reconstruct itself. These experiments, in which rats were continuously administered trientine dihydrochloride in their drinking water so as to achieve constant plasma drug concentrations, showed that the drug thus

administered was unexpectedly effective in reversing the adverse structural and functional effects of diabetic heart disease. Therefore, according to these data, formulations of trientine that maintain constant blood and tissue levels are highly effective in causing removal of systemic copper from the body, and will thus be effective in the treatment of any condition in which pathologically increased tissue copper plays a role in disease initiation or progression. Such diseases include any of the following: heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, as well as Wilson's disease, and forms of cancer.

We recommend a sustained (or slow) release formulation of trientine, trientine dihydrochloride or other pharmaceutically acceptable salts thereof, the composition being suitable for once daily administration yet to provide controlled and long-lasting *in vivo* release.

DOSING REGIMEN FOR TRIENTINE

The half life of trientine, indicated for the treatment and reversal of heart failure and coronary heart disease, is relatively short – being approximately 2 hours. To maintain optimal blood levels, either multiple dose regimen, or a controlled release preparation requiring fewer doses per day is required.

With reference to Figures 36 and 37 there is shown the plasma concentration – time profiles of trientine after oral administration. The plasma concentration was determined using the process as defined in Miyazaki, K., et al., Determination of trientine in plasma of patients with high-performance liquid chromatography. Chem Pharm Bull, 1990. 38:p. 1035-38.

Ideally trientine should be taken in addition to current therapies, at a maximum tolerated dose, utilizing a dose regimen which fits its pharmacokinetic profile. Patients with heart failure and/or coronary artery disease are frequently on multiple drug regimens. Therefore, a controlled release preparation requiring fewer doses per day is preferred. The proof of principle Phase 2 study has shown positive results. However, the dosage regimen was sub-optimal when compared with its pharmacokinetic profile and the study

does not assure the efficacy of the drug which would be required in pivotal trials by regulatory authorities.

The dosage unit if oral preferably delivers not more than 10% trientine dihydrochloride in about 5 hours at an acid pH of about <4.5 and delivers greater than 50% of trientine dihydrochloride in 12 hrs at a pH of about <6.5 in a controlled manner during *in vivo* and *in vitro* dissolution.

Many attempts have been made to develop timed released pharmaceutical preparations which provide a more constant level of an active agent in the blood over hours. Many different approaches have been employed to obtain a timed release.

Coated beads, granules or microspheres containing trientine

A method to achieve modified release of trientine or salts thereof, provided herein is to incorporate the drug into coated beads, granules, or microspheres. Such formulations of trientine have utility for the treatment of diseases in humans and other mammals in which trientine is indicated. In such systems, drug is distributed onto beads, pellets, granules or other particulate systems. Using conventional pan-coating or air-suspension coating techniques, a solution of the drug substance is placed onto small inert nonpareil seeds or beads made of sugar and starch or onto microcrystalline cellulose spheres. The nonpareil seeds are most often in the 425 to 850 micrometer range whereas the microcrystalline cellulose spheres are available ranging from 170 to 600 micrometers (see: Ansel, H. C., Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, p. 232). The microcrystalline spheres are considered more durable during production than sugar-based cores (see: Celphere microcrystalline cellulose spheres. Philadelphia: FMC Corporation, 1996). Methods for manufacture of microspheres suitable for drug delivery have been published (for example, see: Arshady, R. Microspheres and microcapsules: a survey of manufacturing techniques. 1: suspension and cross-linking. Polymer Eng Sci 1989;30:1746-1758; see also, Arshady, R. Microspheres and microcapsules: a survey of manufacturing techniques. 2: coacervation. Polymer Eng Sci 1990;30:905-914; see also: Arshady R. Microspheres and microcapsules: a survey of manufacturing techniques. 3: solvent evaporation. Polymer Eng Sci 1990;30:915-924).

In instances in which the drug dose is large, the starting granules of material may be composed of the drug itself. Some of these granules may remain uncoated to provide immediate drug release. Other granules (about two-thirds to three-quarters) receive varying coats of a lipid material such as beeswax, carnauba wax, glycerylmonostearate, cetyl alcohol, or a cellulose material such as ethylcellulose (infra). Subsequently, granules of different coating thickness are blended to achieve a mixture having the desired drug-release characteristics. The coating material may be coloured with one or more dyes to distinguish granules or beads of different coating thickness (by depth of colour) and to provide distinctiveness to the product. When properly blended, the granules may be placed in capsules or tableted. Various coating systems are commercially available which are aqueous-based and which use ethylcellulose and plasticizer as the coating material (e.g., AquacoatTM [FMC Corporation, Philadelphia] and SurereleaseTM [Colorcon]; for example, see: Aquacoat aqueous polymeric dispersion. Philadelphia: FMC Corporation, 1991; see also: Surerelease aqueous controlled release coating system. West Point, PA: Colorcon, 1990; see also: Butler, J., Cumming, I, Brown, J. et al. A novel multiunit controlled-release system. Pharm Tech 1998;22:122-138; see also: Yazici, E., Oner, L., Kas, H.S. & Hincal, A.A. Phenytoin sodium microspheres: bench scale formulation, process characterization and release kinetics. Pharmaceut Dev Technol 1996;1:175-183). Aqueous-based coating systems eliminate the hazards and environmental concerns associated with organic solvent-based systems. Aqueous and organic solvent-based coating methods have been compared (for example, see: Hogan, J. E. Aqueous versus organic solvent coating. Int J Pharm Tech Prod Manufacture 1982;3:17-20).

The variation in the thickness of the coats and in the type of coating materials used affects the rate at which the body fluids are capable of penetrating the coating to dissolve the drug. Generally, the thicker the coat, the more resistant to penetration and the more delayed will be drug release and dissolution. Typically, the coated beads are about 1 mm in diameter. They are usually combined to have three or four release groups among the more than 100 beads contained in the dosing unit (see: Madan, P. L. Sustained release dosage forms. U. S. Pharmacist 1990;15:39-50). This provides the different desired sustained or extended release rates and the targeting of the coated beads to the desired

segments of the gastrointestinal tract. An example of this type of dosage form is provided by the Spansule™ (SmithKline Beecham Corporation, U.K.).

The following methods may be employed to generate delivery systems containing modified-release delivery forms of trientine or salts thereof, suitable for oral dministration to humans and other mammals.

Two basic mechanisms are available to achieve modified release drug delivery. These are altered dissolution or diffusion of drugs and excipients. Within this context, four processes may be employed, either simultaneously or consecutively. These are as follows: hydration of the device (e.g. swelling of the matrix); diffusion of water into the device; controlled or delayed dissolution of the drug; and controlled or delayed diffusion of dissolved or solubilised drug out of the device. Continuous release is ideally zero-order, and is produced by a constant rate of diffusion or osmosis. Modified release dosage forms commonly fit into one of three categories of system, comprising: monolithic or matrix; reservoir- or membrane-controlled; or osmotic pump systems. Each comprises the following components: active drug; release controlling agents; matrix modifiers; drug modifiers; supplementary coatings; and conventional formulation excipients, such as those described in reference works known to those skilled in the art (for example, see: Kibble A.H (ed.) Handbook of Pharmaceutical Excipients, 3rd Edition, American Pharmaceutical Association, 2000, 665 pp.).

For orally administered dosage forms, extended drug action is achieved by affecting the rate at which the drug is released from the dosage form and/or by slowing the transit time of the dosage form through the gastrointestinal tract (see: Bogner, R. H. Bioavailability and bioequivalence of extended-release oral dosage forms. US Pharmacist 1997;22(Suppl.):3-12). The rate of drug release from solid dosage forms may be modified by the technologies described below which, in general, are based on the following: 1) modifying drug dissolution by controlling access of biologic fluids to the drug through the use of barrier coatings; 2) controlling drug diffusion rates from dosage forms; and 3) chemically reacting or interacting between the drug substance or its pharmaceutical barrier and site-specific biological fluids. Systems by which these objectives are achieved are now described.

In one approach, employing digestion as the release mechanism, the active agent is either coated or entrapped in a substance which is slow digested or dispersed into the intestinal tract. The rate of availability of the active agent is a function of the rate of digestion of the dispersible material. Therefore, the release rate, and thus the effectiveness of the agent, varies from patient to patient depending upon the ability of the patient to digest the material.

In another approach such as disclosed in US Patent No.3247066, the active agent is dispersed in a water-soluble colloid and then coated with a rupturable plastic, non-digestable material which is permeable to the diffusion of water. After ingestion and upon entering the gastrointestinal tract, water in the body fluids diffuses through the coating and causes the colloid to swell. The coating is ruptured by the swelling colloid and the total content of active agent is released. Although there is substantially less variation in the rate of release from patient to patient, substantially all of the active agent is released at once resulting in an initially high blood level content which decreases rapidly with time.

US Patent No. 3115441 discloses another encapsulation method wherein particles of active agent are first given a quick thin coating of a film-forming material and a non-toxic, hydrophobic material and are then coated with successive coatings of an organic solvent-resistant material. The coated particles are mixed with uncoated active agent and this mixture is then formed into a tablet with the coated tablets being entrapped in a matrix of the uncoated active agent. Tablets made according to this method have the advantage of providing immediate delivery because the matrix material (which comprises the initial dosage) dissolves immediately upon ingestion.

A further form of slow release form is any suitable osmotic system where semipermeable membranes of cellulose acetate, cellulose acetate butyrate, cellulose acetate propionate, to control the release of active ingredients. These can be coated with aqueous dispersions of enteric lacquers without changing release rate.

Another approach as in US4025613 to provide an improved blood level profile results from simply applying a film of a non-aqueous solution of cellulose acetate over either individual particles of active agent before tableting or over the outside of tablets

formed from untreated active agent particles, which upon drying forms a coating of cellulose acetate.

Depending on the role attributed to the film-coating, persons skilled in the art will be able to choose the film-forming agent from among the following categories: cellulose derivatives such as hydroxypropylmethylcellulose (HPMC), ethyl cellulose, cellulose acetophthalate, cellulose acetopropionate, cellulose trimelliate, the polymers and copolymers of methacrylic acid and its derivatives. The film-forming agent may be supplemented with: plasticizers (such as polyoxyethylene glycols of high molecular weight, esters of polyacids such as citric acid or phthalic acid) fillers (such as talc, metal oxides such as titanium oxide) colorants chosen from those usable and approved by the pharmaceutical and food industries.

Incorporation of trientine or salts thereof into osmotic pump devices

Further examples of a method by which modified release forms of trientine, suitable for treatment of humans or other mammals, can be produced are provided by the incorporation of trientine into osmotic pump devices. The first notable example of an oral osmotic pump delivery system is that of the Oros™ device developed by Alza Inc. (U.S.A.). This system comprises a core tablet surrounded by a semi-permeable membrane coating having a 0.4 mm diameter hole produced by a laser beam. The core tablet has two layers, once containing the drug (the "active" layer) and the other containing a polymeric osmotic agent (the "push" layer). The core layer consists of active drug, filler, a viscosity modulator, and a solubilizer. The system operates on the principle of osmotic pressure. This system is suitable for delivery of a wide range of drugs, including trientine or salts thereof. The coating technology is straightforward, and release is zero-order.

When the tablet is swallowed, the semi-permeable membrane permits aqueous fluid to enter from the stomach into the core tablet, dissolving or suspending the drug. As pressure increases in the osmotic layer, it forces or pumps the drug solution out of the delivery orifice on the side of the tablet. Only the drug solution (not the undissolved drug) is capable of passing through the hole in the tablet. The system is designed such that only a few drops of water are drawn into the tablet each hour. The rate of inflow of aqueous fluid and the function of the tablet depends on the existence of an osmotic gradient

between the contents of the bi-layer and the fluid in the gastrointestinal tract. Drug delivery is essentially constant as long as the osmotic gradient remains unchanged. The drug release rate may be altered by changing the surface area, the thickness or composition of the membrane, and/or by changing the diameter of the drug release orifice. The drug-release rate is not affected by gastrointestinal acidity, alkalinity, fed conditions, or gut motility. The biologically inert components of the tablet remain intact during gut transit and are eliminated in the faeces as an insoluble shell. Further examples of the application of this technology are provided by Glucotrol XL Extended Release Tablets (Pfizer Inc.) and Procardia XL Extended Release Tablets (Pfizer Inc.; see, Martindale 33rd Ed., p. 2051.3).

Trientine embedded in systems containing slowly eroding or hydrophilic matrix

Further examples of delivery devices that may be employed to deliver trientine or salts of trientine in a form effective for treatment of diseases in humans and other mammals, are provided by those incorporating a hydrophilic polymer matrix, in which trientine is compressed within a mixture of water-swellable hydrophilic polymer. Trientine release occurs as the polymer swells, forming a matrix layer which controls the diffusion of aqueous fluid into the core and thus the rate of diffusion of drug from the system. In such systems, rate of drug release depends upon the tortuous nature of the channels within the gel, and the viscosity of the entrapped fluid. Where such gels are not cross-linked, there is a weaker, non-permanent association between the polymer chains, which relies on secondary bonding. Examples of such matrices include hydroxypropyl methylcellulose (HPMC) and sodium alginate. Advantages of devices formulated from hydrophilic polymer matrix-material include: simplicity and ease of manufacture; the different types of release kinetics that can be achieved (for example, zero-order; first-order combined with pulsatile release); and well-tried technology. With such devices, high loading of active drug is achievable, and effective blending is frequent. Devices contain 20 – 80% of drug (w/w), along with gel modifiers which can enhance drug diffusion; examples of such modifiers include sugars that can enhance the rate of hydration, ions that can influence the content of cross-links, and pH buffers that affect the level of polymer ionization. Hydrophilic matrix devices typically contain pH buffers,

surfactants, counter-ions, lubricants such as magnesium stearate (BP, USP) and a glidant such as colloidal silicon dioxide (USP; colloidal anhydrous silica, BP) in addition to drug substance and hydrophilic matrix.

By this process, the drug substance is combined and made into granules with an excipient that slowly erodes in body fluids, progressively releasing the drug. When these granules are mixed with granules of drug prepared without the excipient, the uncombined granules provide the immediate drug effect whereas the drug-excipient granules provide extended drug action. The granule-mix may be tableted or placed into gelatin capsule shells for oral delivery. Hydrophilic cellulose polymers are commonly used as the excipient-base in tableted matrix systems. The effectiveness of these hydrophilic matrix systems is based on the successive processes of: hydration of the cellulose polymer; gel formation on the polymer's surface; tablet erosion; and the subsequent and continuous release of drug. HPMC (infra), a free-flowing powder, is commonly used to provide the hydrophilic matrix. Tablets are prepared by thoroughly distributing HPMC in the formulation, preparing the granules by wet granulation or roller compaction, and manufacturing the tablets by compression (see: Sheskey, P. J., Cabelka, T. D., Robb, R. T. & Boyce, B. M. Use of roller compaction in the preparation of controlled-release hydrophilic matrix tablets containing methylcellulose and hydroxypropyl methylcellulose polymers. Pharm Tech 1994;18:132-150).

After ingestion, the tablet is wetted by gastric fluid and the polymer begins to hydrate. A gel layer forms around the surface of the tablet and an initial quantity of drug is exposed and released. As water permeates further into the tablet, the thickness of the gel layer is increased and soluble drug diffuses through it. As the outer layer becomes fully hydrated it erodes from the tablet core. If the drug is insoluble, it is released as such with the eroding gel layer. Thus, the rate of drug release is controlled by the processes of diffusion and tablet erosion (see: Formulating for controlled release with Methocel Premium cellulose ethers. Midland, MI: Dow Chemical Company, 1995).

If the bulk density of the tablet is less than one, it is buoyant in the gastric fluids, extending its residence time as it slowly erodes and releases its drug contents (see: Banakar, U. V. Drug delivery systems of the 90s: innovations in controlled release. Am

Pharm 1987;NS27:39-48). An example of this type of product is Valrelease (Roche), a 15-mg slow-release dosage form of diazepam (Valium, Roche). The product is formulated using HBS (Hydrodynamically Balanced Drug-Delivery System), which achieves, in one administration, plasma concentrations of diazepam equivalent to those obtained with conventional Valium 5 mg tablets taken 3-times daily. On contact with gastric fluid, the dosage form demonstrates a bulk density of less than one and thus remains in the stomach for a variable period of time, depending on an individual patient's physiology. Solid dosage forms that have this characteristic have been referred to as floating capsules or tablets (for example, see: Kawashima, Y., Niwa, T., Takeuchi, H, Hino, T. & Itoh, Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharm Sci 1992;82:135-140).

In formulating a successful hydrophilic matrix system, the polymer selected for use must form a gelatinous layer rapidly enough to protect the inner core of the tablet from disintegrating too rapidly after ingestion. As the proportion of polymer is increased in a formulation so is the viscosity of the gel formed with a resulting decrease in the rate of drug diffusion and release (see: Formulating for controlled release with Methocel Premium cellulose ethers. Midland, MI: Dow Chemical Company, 1995). In general, 20% (w/w) of HPMC results in satisfactory rates of drug release for an extended-release tablet formulation. However, as with all formulations, consideration must be given to the possible effects of other formulation ingredients such as fillers, tablet binders, and disintegrants. An example of a proprietary product formulated using a hydrophilic matrix base of HPMC for extended drug release is that of Oramorph SR Tablets (Roxane; see: Martindale 33rd Ed., p. 2014.4).

When hydrophilic matrix formulations are used in the preparation of extended-release capsules, the same concept applies. Following ingestion, aqueous fluid penetrates the capsule shell, contacts the capsule fill, hydrates the outer layer of powder, and forms a gelatinous plug from which the drug content diffuse gradually over time as hydration continues and the gelatinous plug dissolves.

Two-layered tablets can be manufactured, with one layer containing the uncombined drug for immediate release and the other layer having the drug imbedded in a

hydrophilic matrix for extended-release. Three-layered tablets may also be similarly prepared, with both outer layers containing the drug for immediate release. Some commercial tablets are prepared with an inner core containing the extended-release portion of drug and an outer shell enclosing the core and containing drug for immediate release.

Trientine or salts thereof embedded in an inert plastic matrix

A further example of a method of preparation of a device that can accomplish the modified release of trientine or salts of trientine, suitable for oral administration in humans and other mammals, is that of its insertion into an inert plastic matrix. By this method, it is granulated with an inert plastic material such as polyethylene, polyvinyl acetate, or polymethacrylate, and the granulated mixture is then compressed into tablets. Once ingested, the drug is slowly released from the inert plastic matrix by diffusion (for example, see: Bodmeier, R. & Paeratakul, O. Drug release from laminated polymeric films prepared from aqueous latexes. J Pharm Sci 1990;79:32-26; see also, Laghoueg, N., Paulet, J. L., Taverdet, J. L. & Vergnaud, J. M. Oral polymer-drug devices with a core and an erodable shell for constant drug delivery. Int J Pharm 1989;50:133-139; see also, Buckton, G., Efentakis, M., Al-Hmoud, H. & Rajan, Z. The influence of surfactants on drug release from acrylic matrices. Int J Pharm 1991;74:153-158). The compression of the tablet creates the matrix or plastic form that retains its shape during the leaching of the drug and through its passage through the gastrointestinal tract. An immediate-release portion of drug may be compressed onto the surface of the tablet. The inert tablet matrix, expended of drug, is excreted with the faeces. An example of a successful dosage form of this type is Gradumet (Abbott; for example, see: Ferro-Gradumet, Martindale 33rd Ed., p. 1860.4).

Enteric coatings by themselves (if coating a non slow release core) we believe are not an efficient method for the delivery of medications such as trientine dihydrochloride because of the inability of such coating systems to provide or achieve a sustained therapeutic effect after release onset. Enteric coats are designed to dissolve or breakdown in an alkaline environment. The presence of food may increase the pH of the stomach. Therefore, the concurrent administration of enteric-coated trientine dihydrochloride with

food or the presence of food in the stomach may lead to dose dumping and unwanted secondary effects. Furthermore, given the fact that trientine dihydrochloride can give rise to gastrointestinal side-effects, it would be desirable to have a drug delivery system that is capable of providing the controlled delivery of trientine dihydrochloride or other pharmaceutically acceptable salts of trientine in a predictable manner over a long period of time.

Yet another approach is to form a complex between the active agent and an ion exchange resin, whereupon the complex may be tableted, encapsulated or suspended in an aqueous vehicle. Release of the active agent is dependent on the local pH and electrolyte concentration, such that the choice of ion exchange resin may be made so as to preferentially release the active agent in a given region of the alimentary canal.

Delivery devices incorporating trientine complexed to an anion-exchange resin

By way of example a modified release form of trientine can be produced by the incorporation of trientine into complexes with an anion-exchange resin. To produce such complexes, solutions of trientine may be passed through columns containing an ion-exchange resin, forming a complex by the replacement of H_3O^+ ions. The resin-trientine complex is then washed and may be tableted, encapsulated, or suspended in an aqueous vehicle. The release of the trientine is dependent on the pH and the electrolyte concentration in the gastrointestinal fluid. Release is greater in the acidity of the stomach than in the less acidic environment of the small intestine. Alternative examples of this type of extended release preparation are provided by hydrocodone polistirex and chorpheniramine polistirex suspension (Medeva; Tussionex Pennkinetic Extended Release Suspension, see: Martindale 33rd Ed., p. 2145.2) and by phentermine resin capsules (Pharmanex; Ionamin Capsules see: Martindale 33rd Ed., p.1916.1).

Such systems for modified-release delivery of trientine can incorporate polymer barrier coating and bead technologies in addition to the ion-exchange mechanism. The initial trientine dose comes from an uncoated portion, and the remainder from the coated beads. The coating does not dissolve, and release may be extended over a 12 h period by ion exchange. The drug-containing particles are minute, and may be also suspended to produce a liquid with extended-release characteristics, as well as solid dosage forms.

Such preparations may also be suitable for administration, for example in depot preparations suitable for intramuscular injection.

Microencapsulated trientine

A further method to produce modified release preparations of trientine or salts thereof, provided herein is that of microencapsulation. Such microencapsulated preparations of trientine are useful for the treatment of humans and other mammals, in whom trientine therapy is indicated. Microencapsulation is a process by which solids, liquids or even gasses may be encapsulated into microscopic size particles through the formation of thin coatings of "wall" material around the substance being encapsulated such as disclosed iun US Patent Nos. 3,488,418; 3,391,416 and 3,155,590. Gelatin (BP, USP) is commonly employed as a wall-forming material in microencapsulated preparations, but synthetic polymers such as polyvinyl alcohol (USP), ethylcellulose (BP, USP), polyvinyl chloride, and other materials may also be used (for example, see: Zentner, G. M., Rork, G. S. & Himmelstein, K. J. Osmotic flow through controlled porosity films: an approach to delivery of water soluble compounds. J Controlled Release 1985;2:217-229; see also: Fites, A. L., Banker, G. S. & Smolen, V. F. Controlled drug release through polymeric films. J Pharm Sci 1970;59:610-613; see also: Samuelov, Y., Donbrow, M. & Friedman, M. Sustained release of drugs from ethylcellulose-polyethylene glycol films and kinetics of drug release. J Pharm Sci 1979;68:325-329).

Encapsulation begins with the dissolving of the prospective wall material, say gelatin, in water. Trientine is then added and the two-phase mixture is thoroughly stirred. With the material to be encapsulated broken up to the desired particle size, a solution of a second material is added, preferably acacia (BP, USP). This additive material is chosen to have the ability to concentrate the gelatin (polymer) into tiny liquid droplets. These droplets (the coacervate) then form a film or coat around the particles of the solid trientine as a consequence of the extremely low interfacial tension of the residual water or solvent in the wall material so that a continuous, tight, film-coating remains on the particle (see: Ansel, H. C., Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, p. 233). The final dry microcapsules are free-flowing, discrete particles of coated material. Of the total particle weight, the wall

material usually represents between 2 and 20% (w/w). The coated particles are then admixed with tableting excipients and formed into dosage sized tablets. Different rates of trientine release may be obtained by changing the core-to-wall ratio, the polymer used for the coating, or the method of microencapsulation (for example, see: Yazici, E., Oner, L., Kas, H.S. & Hincal, A.A. Phenytoin sodium microspheres: bench scale formulation, process characterization and release kinetics. Pharmaceut Dev Technol 1996;1:175-183).

One of the advantages of microencapsulation is that the administered dose of trientine is subdivided into small units that are spread over a large area of the gastrointestinal tract, which may enhance absorption by diminishing localized drug concentrations (see: Yazici et al., supra). An example of a drug that is commercially available in a microencapsulated extended-release dosage form is potassium chloride (Micro-K Exten-caps, Wyeth-Ayerst, Martindale 33rd Ed., p1968.1).

Delivery devices incorporating trientine complexed with one or more suitable anions

Further examples of a method by which modified release forms of trientine, suitable for treatment of humans or other mammals, can be produced are provided by the incorporation of trientine into certain complexes such as those with various forms of tannic acid (for example, see: Merck Index 12th Ed., 9221). Drug substances such as trientine, when chemically combined with other chemical agents such as anions formed from various forms of tannic acid, constitute complexes that are only slowly soluble in body fluids, depending for example on the pH of the environment. This slow dissolution rate provides for the extended release of the drug. For example, trientine salts of tannic acid, trientine tannates, provide for this quality, and are expected to possess utility for the treatment of conditions in which increased copper plays a role. Examples of equivalent products are provided by those having the tradename Rynatan (Wallace: for example, see: Madan, P. L. Sustained release dosage forms. U. S. Pharmacist 1990;15:39-50; see also: Ryna-12 S, which contains a mixture of mepyramine tannate with phenylephrine tannate, Martindale 33rd Ed., 2080.4).

Repeat action tablets containing trientine or salts thereof.

Further examples of a method by which modified release forms of trientine, suitable for treatment of humans or other mammals, can be produced are provided by the

incorporation of trientine into repeat action tablets. These are prepared so that an initial dose of the drug is released immediately followed later by a second dose. The tablets may be prepared with the immediate-release dose in the tablet's outer shell or coating with the second dose in the tablet's inner core, separated by a slowly permeable barrier coating. In general, the drug from the inner core is exposed to body fluids and released 4 to 6 hours after administration. An example of this type of product is proved by Repetabs (Schering Inc.). Repeat action dosage forms are suitable for the administration of trientine for chronic conditions such as heart failure, diabetic heart disease, acute coronary syndrome, hypertensive heart disease, ischaemic heart disease, coronary artery disease, peripheral arterial disease, Wilson's disease, or any form of cancer. This form of delivery is particularly suitable for delivery of trientine, since it has a rapid rate of absorption and excretion.

Delayed-release oral dosage forms containing trientine or salts thereof

Further examples of methods suitable for the preparation of modified release forms of trientine or its salts suitable for oral administration to humans or other mammals are provided by its incorporation into delivery devices in which drug is contained in tablets or capsules that are enteric coated. The release of trientine from an oral dosage form can be intentionally delayed until it reaches the intestines. This form of delivery conveys the advantage of minimizing the gastric irritation that may be caused in some patients by trientine. The enteric coating may be pH-dependent breaking down in the less acidic environment of the intestine, time-dependent and eroding by moisture over time during gastrointestinal transit, or enzyme-dependent and deteriorating due to the hydrolysis-catalyzing action of intestinal enzymes (for example, see: Muhammad, N. A., Boisvert, W., Harris, M. R. & Weiss, J. Modifying the release properties of Eudragit L30D. Drug Dev Ind Pharm. 1991;17:2497-2509). Among the many agents used to enteric coat tablets and capsules known to those skilled in the art are fats including triglycerides, fatty acids, waxes, shellac, and cellulose acetate phthalate. Further examples of enteric coated preparations are provided in the USP (supra).

Delivery devices incorporating trientine in a monolithic matrix

Further examples of methods suitable for the preparation of modified release forms of trientine or its salts suitable for oral administration to humans or other mammals are provided by incorporation into delivery devices in which trientine is incorporated into a monolithic matrix. Examples of useful systems include those in which trientine is incorporated into a polymer matrix (see: Davis, S. S. & Illum, L. Polymeric microspheres as drug carriers. Biomaterials 1988;9:111-115; see also: Douglas, S. J., Davis, S. S. & Illum, L. Nanoparticles in drug delivery. C. R. C. Crit Rev Therap Drug Carrier Syst 1987;3:233-261: see also: Oppenheim, R. C. Solid colloidal drug delivery systems: nanoparticles. Int J Pharm 1981;8:217-234). Other useful approaches include those in which the drug is incorporated into polymeric colloidal particles or microencapsulates (microparticles, microspheres or nanoparticles) in the form or reservoir and matrix devices (see: Douglas et al., supra; see also: Oppenheim, supra; see also, Higuchi, T. Mechanism of sustained action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963;52:1145-1149). Further useful approaches have drug incorporated in pendent attachments to a polymer matrix (for example, see: Scholsky, K. M. & Fitch, R. M. Controlled release of pendant bioactive materials from acrylic polymer colloids. J Controlled Release 1986;3:87-108). In these devices, drugs are attached by means of an ester linkage to poly(acrylate) ester latex particles prepared by aqueous emulsion polymerization. Further embodiments incorporate dosage forms in which the drug is bound to a biocompatible polymer by a labile chemical bond e.g. polyanhydrides prepared from a substituted anhydride (itself prepared by reacting an acid chloride with the drug: methacryloyl chloride and the sodium salt of methoxy benzoic acid) have been used to form a matrix with a second polymer (Eudragit RL) which releases drug on hydrolysis in gastric fluid (see: Chafi, N., Montheard, J. P. & Vergnaud, J. M. Release of 2-aminothiazole from polymeric carriers. Int J Pharm 1992;67:265-274). Monolithic matrix devices comprise those formed using either of the following systems. (I), drug particles are dispersed in a soluble matrix, in which they become increasingly available as the matrix dissolves or swells; examples include hydrophilic colloid matrices, such as hydroxypropylcellulose (BP) or hydroxypropyl

cellulose (USP); hydroxypropyl methylcellulose (HPMC; BP, USP); methylcellulose (MC; BP, USP); calcium carboxymethylcellulose (Calcium CMC; BP, USP); acrylic acid polymer or carboxy polymethylene (Carbopol) or Carbomer (BP, USP); or linear glycuronan polymers such as alginic acid (BP, USP), for example those formulated into microparticles from alginic acid (alginate)-gelatin hydrocolloid coacervate systems, or those in which liposomes have been encapsulated by coatings of alginic acid with poly-L-lysine membranes. (II), drug particles are dissolved in an insoluble matrix, from which drug becomes available as solvent enters the matrix, often through channels, and dissolves the drug particles. Examples include systems formed with a lipid matrix, or insoluble polymer matrix, including preparations formed from Carnauba wax (BP; USP); medium-chain triglyceride such as fractionated cocoanut oil (BP) or triglycerida saturata media (PhEur); or cellulose ethyl ether or ethylcellulose (BP, USP). Lipid matrices are simple and easy to manufacture, and incorporate the following blend of powdered components: lipids (20-40% hydrophobic solids w/w) which remain intact during the release process; drug substance; channeling agent, such as sodium chloride or sugars, which leaches from the formulation, forming aqueous micro-channels (capillaries) through which solvent enters, and through which drug is released. In the alternative system, which employs an insoluble polymer matrix, drug is embedded in an inert soluble polymer and is released by leaching of aqueous fluid, which diffuses into the core of the device through capillaries formed between particles, and from which drug diffuses out of the device. The rate of release is controlled by degree of compression, particle size, and the nature and relative content (w/w) of excipients. An example of such a device is that of Ferrous Gradumet (Martindale 33rd Ed., 1360.3).

Drug delivery devices incorporating trientine in a membrane-control system

Yet further examples of methods suitable for the preparation of modified release forms of trientine or its salts suitable for oral administration to humans or other mammals are provided by incorporation into delivery devices in which drug is incorporated into a membrane-control system. Such devices comprise a rate-controlling membrane surrounding a drug reservoir. Following oral administration the membrane gradually becomes permeable to aqueous fluids, but does not erode or swell. The drug reservoir

may be composed of a conventional tablet, or a microparticle pellet containing multiple units which do not swell following contact with aqueous fluids. The cores dissolve without modifying their internal osmotic pressure, thereby avoiding the risk of membrane rupture, and typically comprise 60:40 mixtures of lactulose: microcrystalline cellulose (w/w). Drug is released through a two-phase process, comprising diffusion of aqueous fluids into the matrix, followed by diffusion of drug out of the matrix. Multiple-unit membrane-controlled systems typically comprise more than one discrete unit. They can contain discrete spherical beads individually coated with rate-controlling membrane and may be encapsulated in a hard gelatin shell (examples of such preparations include Contac 400; Martindale 33rd Ed., 1790.1 and Feospan; Martindale 33rd Ed., p.1859.4). Alternatively, multiple-unit membrane-controlled systems may be compressed into a tablet (for example, Suscard; Martindale 33rd Ed., p.2115.1). Alternative implementations of this technology include devices in which the drug substance is coated around inert sugar spheres, and devices prepared by extrusion spheronization employing a conventional matrix system. Advantages of such systems include the more consistent gastro-intestinal transit rate achieved by multiple-unit systems, and the fact that such systems infrequently suffer from catastrophic dose dumping. They are also ideal for the delivery of more than one drug at a time.

We prefer for oral delivery a sustained release form which is a matrix formation, such a matrix formation taking the form of film coated spheroids containing as active ingredient trientine dihydrochloride and a non water soluble spheronising agent. The term "spheroid" is known in the pharmaceutical art and means spherical granules having a diameter usually of between 0.01 mm and 4 mm.

The spheronising agent may be any pharmaceutically acceptable material that, together with the active ingredient, can be spheronised to form spheroids. Microcrystalline cellulose is preferred.

A suitable microcrystalline cellulose is, for example, the material sold as Avicel PH 101 (Trade Mark, FMC Corporation). According to a preferred aspect of the present invention, the film coated spheroids contain between 70% and 99% (by wt), especially

between 80% and 95% (by wt), of the spheronising agent, especially microcrystalline cellulose.

In addition to the active ingredient and spheronising agent, the spheroids may also contain a binder. Suitable binders, such as low viscosity, water soluable polymers, will be well known to those skilled in the pharmaceutical art. A suitable binder is, in particular polyvinylpyrrolidone in various degrees of polymerization. However, water soluble hydroxy lower alkyl celluloses, such as hydroxy propyl cellulose, are preferred. Additionally (or alternatively) the spheroids may contain a water insoluble polymer, especially an acrylic polymer, an acrylic copolymer, such as a methacrylic acid-ethyl acrylate copolymer, or ethyl cellulose.

Other thickening agents or binders include:

the lipid type, among which are vegetable oils (cotton seed, sesame and groundnut oils) and derivatives of these oils (hydrogenated oils such as hydrogenated castor oil, glycerol behenate,

the waxy type such as natural carnauba wax or natural beeswax, synthetic waxes such as cetyl ester waxes,

the amphiphilic type such as polymers of ethylene oxide (polyoxyethylene glycol of high molecular weight between 4000 and 100000) or propylene and ethylene oxide copolymers (poloxamers),

the cellulosic type (semisynthetic derivatives of cellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, hydroxymethylcellulose, of high molecular weight and high viscosity, gum) or any other polysaccharide such as alginic acid,

the polymeric type such as acrylic acid polymers (such as carbomers), and

the mineral type such as colloidal silica, bentonite

Also included in the sustained dosage forms in accordance with the present invention are any variants of the oral forms that are adapted for suppository use.

When rectally administered in the form of suppositories, these compositions may be prepared by mixing the trientine moiety with a suitable non-irritating excipient, such as

cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

Suitable diluents for the active ingredient in the pellets, spheroids or core are eg, microcrystalline cellulose, lactose, dicalcium phosphate, calcium carbonate, calcium sulphate, sucrose, dextrates, dextrin, dextrose, dicalcium phosphate dihydrate, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, cellulose, microcrystalline cellulose, sorbitol, starches, pregelatinized starch, talc, tricalcium phosphate and lactose. Suitable lubricants are eg. magnesium stearate and sodium stearyl fumarate. Suitable binding agents are eg, hydroxypropyl methyl cellulose, polyvidone and methyl cellulose.

Suitable binders that may be included are: gum arabic, gum tragacanth, guar gum, alginic acid, sodium alginate, sodium carboxymethylcellulose, dextrin, gelatin, hydroxyethylcellulose, hydroxypropylcellulose, liquid glucose, magnesium and aluminium silicate, maltodextrin, povidone, pregelatinized starch, starch and zein.

Suitable disintegrating agents are starch, sodium starch glycolate, crospovidone and croscarmalose sodium. Suitable surface active are Poloxamer 188®, polysorbate 80 and sodium lauryl sulfate. Suitable flow aids are talc colloidal anhydrous silica. Suitable lubricants that may be used are glidants (such as anhydrous silicate, magnesium trisilicate, magnesium silicate, cellulose, starch, talc or tricalcium phosphate) or alternatively antifriction agents (such as calcium stearate, hydrogenated vegetable oils, paraffin, magnesium stearate, polyethylene glycol, sodium benzoate, sodium lauryl sulphate, fumaric acid, stearic acid or zinc stearate and talc). Suitable water soluble polymers are PEG with molecular weights in the range 1000 to 6000.

Delayed release through the use of a tablet, pellet, spheroid or core itself, which besides having a filler and binder, other ancillary substances, in particular lubricants and nonstick agents, and disintegrants. Examples of lubricants and nonstick agents which may be mentioned are higher fatty acids and their alkali-metal and alkaline-earth-metal salts, such as calcium stearate. Suitable disintegrants are, in particular, chemically inert agents. Disintegrants which may be mentioned as preferred are cross linked polyvinylpyrrolidone, cross linked sodium carboxymethylcelluloses and sodium starch glycolate.

Multi tablets include small spheroid-shaped compressed minitables that may have a diameter of between 3 to 4 mm and can be placed in gelatin capsule shell to provide the desired pattern of drug release. Each capsule may contain 8-10 minitablets, some uncoated for immediate release and others coated for extended drug release.

The granules employed may be coated or receive varying coats of lipid material, where those granules not receiving a coating provide immediate drug release. Granules of different coating thickness can be blended to achieve a mix of different granules having the desired drug release characteristics.

Examples of film-forming polymers which can be used in the water-insoluble release-slowing intermediate layer(s) (to be applied to a pellet, spheroid or tablet core) include ethylcellulose, polyvinyl acetate, Eudragit® RS, Eudragit® RL, etc. (Each of Eudragit® RS and Eudragit® RL is an ammonio methacrylate copolymer.) The release rate can be controlled not only by incorporating therein suitable water-soluble pore formers, such as lactose, mannitol, sorbitol, etc., but also by the thickness of the coating layer applied.

Also within the scope of the present invention are preparations that are analogous to those disclosed in, for example, US Patents 4,968,505, 5,874,107, 6,312,724 which disclose respectively long acting preparations where the active ingredient coupled with an organic acid is coated with a sustained release coat, a sustained release tablet and a matrix tablet but of course in each instance substituting the trientine salt for the diclophenic sodium. Other delivery systems will also be applicable to achieve the outcome desired.

Controlled release from a matrix of embedded or capsulated discrete solid particles as disclosed in WO 98/18610 (Van Lengerich) (indicated as being suitable for an extensive list of active ingredients (including trientine hydrochloride)) we believe may have application as, or as an inclusion in, any of the sustained release dosage forms of the present invention.

Another approach is to embed the active agent in a slowly eroding or hydrophilic matrix, wherein the active agent is combined granulated with an excipient material that slowly erodes in bodily fluids and so progressively releases the active agent for absorption. The granules may be tableted or encapsulated for delivery. Commonly,

hydrophilic cellulose polymers, such as hydroxypropyl methylcellulose, are used as the excipient matrix material.

A further approach to a slow release form is to embed the active agent in an inert plastics matrix. The active agent is combined and granulated with an inert plastic materials, such a polyethylene, polyvinyl acetate, polymethacrylate or similar polymer, and compressed into tablets and the active agent is released from the matrix by diffusion.

Non-oral modified-release systems containing trientine

Examples of methods suitable for the preparation of modified release forms of trientine or its salts suitable for non-oral administration to humans or other mammals are provided by incorporation into delivery devices, in which drug is incorporated into dosage forms designed to provide extended drug action. Extended drug action may be achieved through a modification of the physical or chemical characteristics of the drug substance, a change in the characteristics of the drug carrier or vehicle, or by the fabrication of rate-controlled drug delivery systems.

Formulations of trientine or salts thereof suitable for administration through transdermal drug delivery systems, including ionophoresis or sonophoresis

Yet further embodiments of the invention include forms of trientine or salts thereof incorporated into transdermal drug delivery systems, such as those described in: Transdermal Drug Delivery Systems, Chapter 10. In: Ansel, H. C., Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, pp. 263 - 278). Transdermal drug delivery systems facilitate the passage of therapeutic quantities of drug substances through the skin and into the systemic circulation to exert systemic effects, as originally described (see: Stoughton, R. D. Percutaneous absorption. Toxicol Appl Pharmacol 1965;7:1-8). Evidence of percutaneous drug absorption may be found through measurable blood levels of the drug, detectable excretion of the drug and/or its metabolites in the urine, and through the clinical response of the patient to its administration. For transdermal drug delivery, it is considered ideal if the drug penetrates through the skin to the underlying blood supply without drug build up in the dermal layers (Black, C. D. Transdermal drug delivery systems. US Pharm 1982;1:49). Formulations of drugs suitable for trans-dermal delivery are known to those

skilled in the art, and are described in references such as Ansel et al. (supra). Methods known to enhance the delivery of drugs by the percutaneous route include chemical skin penetration enhancers, which increase skin permeability by reversibly damaging or otherwise altering the physicochemical nature of the stratum corneum to decrease its resistance to drug diffusion (see: Shah, V. P., Peck, C. C. & Williams, R. L. Skin penetration enhancement: clinical pharmacological and regulatory considerations. In: Walters, K. A. & Hadgraft, J. (Eds.) Pharmaceutical skin penetration enhancement. New York: Dekker, 1993). Among effective alterations are increased hydration of the stratum corneum and/or a change in the structure of the lipids and lipoproteins in the intercellular channels brought about through solvent action or denaturation (see: Walters KA. Percutaneous absorption and transdermal therapy. Pharm Tech 1986;10:30-42). Skin penetration enhances suitable for formulation with trientine in Transdermal Drug Delivery Systems may be chosen from the following list: acetone, laurocapram, dimethylacetamide, dimethylformamide, dimethylsulphoxide, ethanol, oleic acid, polyethylene glycol, propylene glycol and sodium lauryl sulphate. Further skin penetration enhancers may be found in publications known to those skilled in the art (for example, see: Osborne, D. W., Henke, J. J. Skin penetration enhancers cited in the technical literature. Pharm Tech 1997:21:50-66; Rolf, D. Chemical and physical methods of enhancing transdermal drug delivery. Pharm Tech 1988;12:130-139).

In addition to chemical means, there are physical methods that enhance transdermal drug delivery and penetration; these include iontophoresis and sonophoresis. Iontophoresis involves the delivery of charged chemical compounds across the skin membrane using an applied electrical field. Such methods have proven suitable for delivery of a number of drugs. Accordingly, another embodiment of the invention comprises trientine or salts thereof formulated in such a manner as to be suitable for administration by iontophoresis. In yet a further embodiment of the invention is provided formulations of trientine or salts thereof suitable for administration by sonophoresis. Formulations of trientine suitable for administration by iontophoresis or sonophoresis may be in the form of gels, creams, or lotions.

FORMULATIONS OF TRIENTINE OR SALTS THEREOF SUITABLE FOR ADMINISTRATION THROUGH TRANSDERMAL DRUG DELIVERY SYSTEMS, INCLUDING IONTOPHORESIS OR SONOPHORESIS

Transdermal delivery may utilize, among others, monolithic delivery systems drug-impregnated adhesive delivery systems (eg. the Latitude™ drug-in-adhesive system from 3M), active transport devices and membrane-controlled systems. Monolithic systems incorporate an active agent matrix, comprising a polymeric material in which the active agent is dispersed between backing and frontal layers. Drug impregnated adhesive delivery systems comprise an adhesive polymer in which the active agent and any excipients are incorporated into the adhesive polymer. Active transport devices incorporate an active agent reservoir, often in liquid or gel form, a membrane that may be rate controlling, and a driving force to propel the active agent across the membrane. Membrane-controlled transdermal systems commonly comprise an active agent reservoir, often in liquid or gel form, a membrane that may be rate-controlling and backing, adhesive and/or protecting layers.

Transdermal delivery dosage forms include those which substitute the trientine active ingredient, preferably trientine dihydrochloride for the diclofenic or other pharmaceutically acceptable salt thereof referred to in the transdermal delivery systems disclosed in, by way of example, US Patents 6,193,996, 6,262,121, the full content of which is hereby here included.

Delayed-release ocular preparations containing trientine or salts thereof

Disease of the retinal arteries, leading to leading to leakage of plasma and ultimately to diabetic retinopathy, is a leading cause of impaired vision and blindness consequent upon diabetes. Trientine therapy is effective in treating diabetic arterial disease. This aspect of the invention provides ocular preparations of trientine suitable for administration to humans for the treatment of disease of the retinal arteries in diabetes. Such administration is expected to yield high localized concentrations of drug, suitable for treatment of diabetic arterial disease in the retina, and diabetic retinopathy.

One of the problems associated with the use of ophthalmic solutions is the rapid loss of administered drug due to blinking of the eye and the flushing effect of lacrimal fluids. Up to 80% of an administered dose may be lost through tears and the action of nasolacrimal drainage within 5 minutes of installation. Extended periods of therapy may be achieved by formulations which increase the contact time between the medication and the corneal surface. This may be accomplished through use of agents that increase the viscosity of solutions; by ophthalmic suspensions in which the drug particles slowly dissolve; by slowly dissipating ophthalmic ointments; or by use of ophthalmic inserts. Preparations of trientine or its salts suitable for ocular administration to humans may be formulated using synthetic high molecular weight cross-linked polymers such as those of acrylic acid (e.g. Carbopol 940) or gellan gum (Gelrite; see, Merck Index 12th Ed., 4389), a compound that forms a gel upon contact with the precorneal tear film (e.g. as employed in Timoptic-XE by Merck, Inc.).

Further embodiments of the invention include delayed-release ocular preparations containing trientine in ophthalmic inserts, such as the OCUSERT system (Alza Inc.). Typically, such inserts are elliptical with dimensions of about 13.4 mm by 5.4 mm by 0.3 mm (thickness). The insert is flexible and has a drug-containing core surrounded on each side by a layer of hydrophobic ethylene/vinyl acetate copolymer membranes through which the drug diffuses at a constant rate. The white margin around such devices contains white titanium dioxide, an inert compound that confers visibility. The rate of drug diffusion is controlled by the polymer composition, the membrane thickness, and the drug solubility. During the first few hours after insertion, the drug release rate is greater than that which occurs thereafter in order to achieve initially therapeutic drug levels. The trientine-containing inserts may be placed in the conjunctival sac from which they release their medication over a typical 7-d period in the treatment of diabetic retinal disease.

A further embodiment is another form of ophthalmic insert formed as a rod shaped, water soluble structure composed of hydroxypropyl cellulose in which trientine is embedded. The insert is placed into the inferior cul-de-sac of the eye once or twice daily in the treatment of diabetic retinal disease. The inserts soften and slowly dissolve,

releasing the drug which is then taken up by the ocular fluids. A further example of such a device is furnished by Lacrisert (Merck Inc.).

Topical administration of the trientine active ingredient can be prepared as an admixture or other pharmaceutical formulation to be applied in a wide variety of ways including, but are not limited to, lotions, creams gels, sticks, sprays, ointments and pastes. These product types may comprise several types of formulations including, but not limited to solutions, emulsions, gels, solids, and liposomes. If the topical composition is formulated as an aerosol and applied to the skin as a spray-on, a propellant may be added to a solution composition. Suitable propellants as used in the art can be utilized. By way of example of topical administration of an active agent, reference is made to US Patents 5,602,125, 6,426,362 and 6,420,411.

Suppositories are generally solid dosage forms intended for insertion into body orifices including rectal, vaginal and occasionally urethrally and can be long acting or slow release. Suppositories include a base that can include, but is not limited to, materials such as alginic acid, which will prolong the release of the pharmaceutically acceptable active ingredient over several hours (5-7). Such bases can be characterized into two main categories and a third miscellaneous group: 1) fatty or oleaginous bases, 2) water-soluble or water-miscible bases and 3) miscellaneous bases, generally combinations of lipophilic and hydrophilic substances. Fatty or oleaginous bases include hydrogenated fatty acids of vegetable oils such as palm kernel oil and cottonseed oil, fat-based compound containing compounds of glycerin with the higher molecular weight fatty acids such as palmitic and stearic acids, cocoa butter is also used where phenol and chloral hydrate lower the melting point of cocoa butter when incorporated, solidifying agents like cetyl esters wax (about 20%) or beeswax (about 4%) may be added to maintain a solid suppository. Other bases include other commercial products such as Fattibase (triglycerides from palm, palm kernel and coconut oils with self-emulsifying glycerol monostearate and poloxyl stearate), Wecobee and Witepsol bases.

Water-soluble and Water-miscible bases are generally glycerinated gelatin and base of polyethylene glycols. The miscellaneous bases include mixtures of the oleaginous and

water-soluble or water-miscible materials. An example of such a base in this group is polyoxyl 40 stearate and polyoxyethylene diols and the free glycols.

Transmucosal delivery may utilize any mucosal membrane but commonly utilizes the nasal, buccal, vaginal and rectal tissues. Formulations suitable for nasal administration may be administered in a liquid form, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, including aqueous or oily solutions of the active ingredient. Formulations for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, of less than about 100 microns, preferably less than about 50 microns, which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Compositions in solution may be neubulised by the use of inner gases and such nebulised solutions may be breathed directly from the neulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solutions, suspensions or powder compositions may be administered, preferably orally or nasally from devices which deliver the formulation in an appropriate manner. Formulations may be prepared as aqueous solutions example in saline, solutions employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bio-availability, fluorocarbons, and/or other solubilising or dispersing agents known in the art.

For rectal administration, the subject compositions may be provided as suppositories, as solutions for enemas, or other convenient application.

Formulation for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, inserts, implants or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

The pharmaceutically acceptable active agent of trientine dihydrochloride can be administered in the *form* of a depot injection which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly. The pellets, or cylinders may additionally be coated with a suitable biodegradable polymer chosen so as to provide a desired release profile. The active

ingredient may alternatively be micropelleted. Active agent micropellets using bioacceptable polymers can be designed to allow release rates to be manipulated to provide a desired release profile.

Alternatively, injectable depot forms can be made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations can also be prepared by entrapping the drug in liposomes, examples of which include unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearyl aamine or phosphatidylcholines. Depot injectable formulations can also be prepared by entrapping the drug in microemulsions which are compatible with body tissue. By way of example reference is made to US Patent Applications 6,410,041 and 6,362,190.

Depot injection may be performed whereby the active ingredient can be compressed into pallet(s) or small cylinders or where the active ingredient can be microencapsulated in a matrix of biodegradable polymers such as polylactide –polyglycolide, polyorthoesters, polyanhydrids, or maybe entrapped in liposomes or microemulsions compatible with body tissue.

Implantable infusion devices may employ inert material such as biodegradable polymers listed above or synthetic silicones for example cylastic, silicone rubber or other polymers manufactured by the Dow-Corning Corporation. The polymer may be loaded with active agent and any excipients. Implantable infusion devices may also comprise a coating of, or a portion of, a medical device wherein the coating comprises the polymer loaded with active agent and any excipient.

Such an implantable infusion device may be prepared as disclosed in US Patent 6309380 by coating the device with an in vivo biocompatible and biodegradable or bioabsorbable or bioerodable liquid or gel solution containing a polymer with the solution comprising a desired dosage amount of active ingredient and any excipients. The solution is converted

to a film adhering to the medical device thereby forming the implantable drug-deliverable medical device.

An implantable infusion device may also be prepared by the in situ formation of an active agent containing solid matrix as disclosed in US Patent 6120789, herein incorporated in it's entirety.

Implantable infusion devices may be passive or active. An active implantable infusion device may comprise an active agent reservoir, a means of allowing the active agent to exit the reservoir, for example a permeable membrane, and a driving force to propel the active agent from the reservoir. Such an active implantable infusion device may additionally be activated by an extrinsic signal, such as that disclosed in WO 02/45779, wherein the implantable infusion device comprises a system configured to deliver active agent comprising an external activation unit operable by a user to request activation of the implantable infusion device, including a controller to reject such a request prior to the expiration of a lockout interval. Alternatively, implantable infusion devices may employ liposome delivery systems such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles can be formed from a variety of phospholipids, such as cholesterol, stearyl amine or phosphatidylcholines.

Targeted release delivery systems where the active agent is isolated or concentrated in a body region, tissue or site for absorption or action.

Extended-release formulations containing trientine or salts thereof suitable for parenteral administration

Extended rates of drug action following injection may be achieved in a number of ways, including the following: crystal or amorphous drug forms having prolonged dissolution characteristics; slowly dissolving chemical complexes of the drug entity; solutions or suspensions of drug in slowly absorbed carriers or vehicles (as oleaginous); increased particle size of drug in suspension; or, by injection of slowly eroding microspheres of drug (for example, see: Friess, W., Lee, G. and Groves, M. J. Insoluble collagen matrices for prolonged delivery of proteins. Pharmaceut Dev Technol 1996;1:185-193). The duration of action of the various forms of insulin for example is

based in part on its physical form (amorphous or crystalline), complex formation with added agents, and its dosage form (solution of suspension).

In addition to the above means of achieving extended drug action, the rate and duration of drug delivery may be controlled by slow intravenous or subcutaneous infusion, using mechanically-controlled drug infusion pumps [refer here to related filing of parenteral formulations of trientine].

Further examples of parenteral products with long-acting properties are available from the USP (supra).

Yet further embodiments of the invention include the incorporation of trientine or its salts into suppositories (rectal or vaginal), or into vaginal inserts.

We propose as a consequence of the studies undertaken, that equate human copper values depletion against those of the STZ rat, a dosage form which will perform sufficiently as shown by the Figure 3 studies reliant on a dose peak much lower (perhaps the order of 10) than that delivered orally for the treatment of Wilson's disease in fast release oral dosage forms taken twice daily or as otherwise taken in accordance with the SYPRINE® data sheet.

DATED THIS 20th DAY OF August 2002
AJ PARK
PER J. Finlay
AGENTS FOR THE APPLICANT

Confocal microscope images of Rat left ventricle

Stain: Phalloidin (f-actin) and Lewis (β_1-integrin)

13 wk Sham | 13 wk STZ | 13 wk STZ / 7 wk Drug

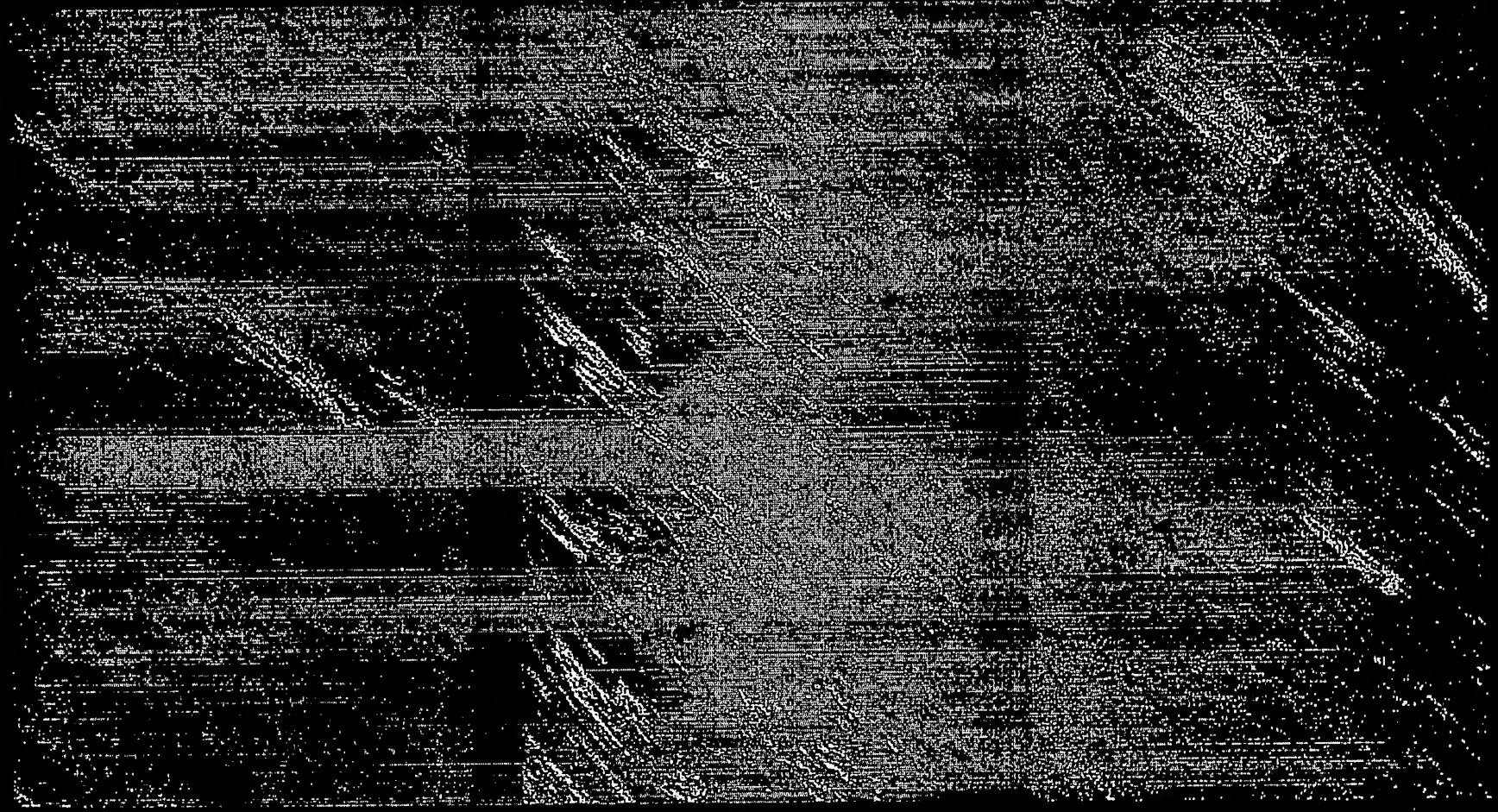


Figure 1A

630 x magnification

Confocal microscope images of Rat left ventricle

Stain: Phalloidin (f-actin) and Lewis (β_1-integrin)

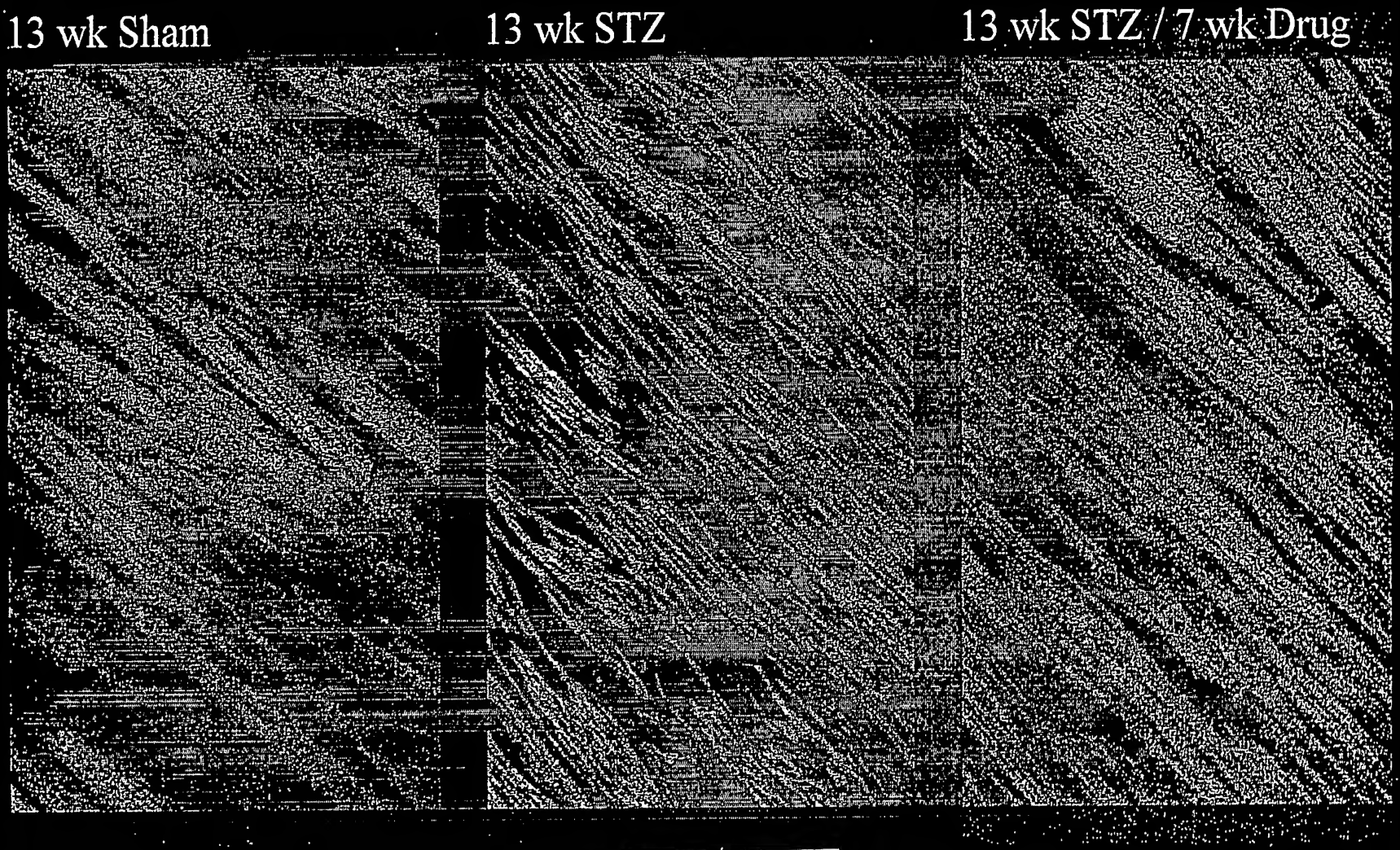
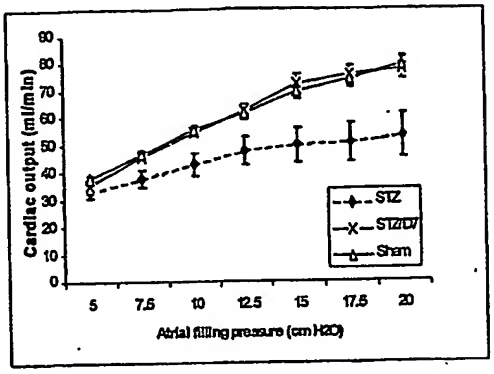


Figure 1B

630 x magnification

Trientine Restores Normal Heart Function



Cardiac output in response to increasing preload with GC811007 treatment from weeks 7-13

STZ/D7 v STZ = $p<0.03$; STZ/D7 v Sham = ns; STZ v Sham = all $p<0.05$

FIGURE 2A

Functional Survival of Working Hearts

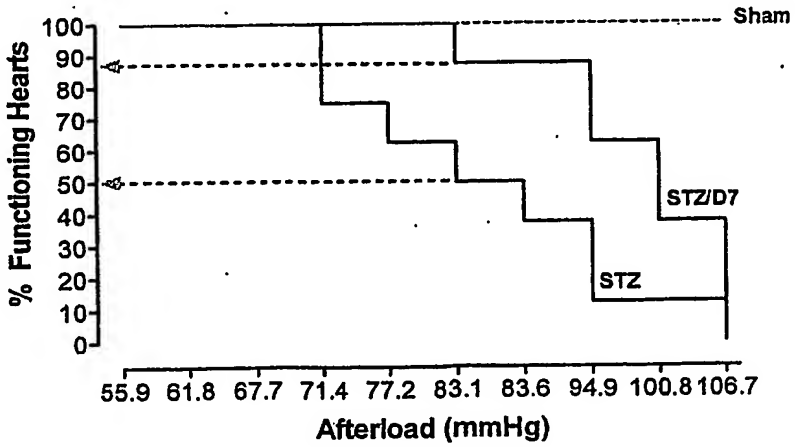
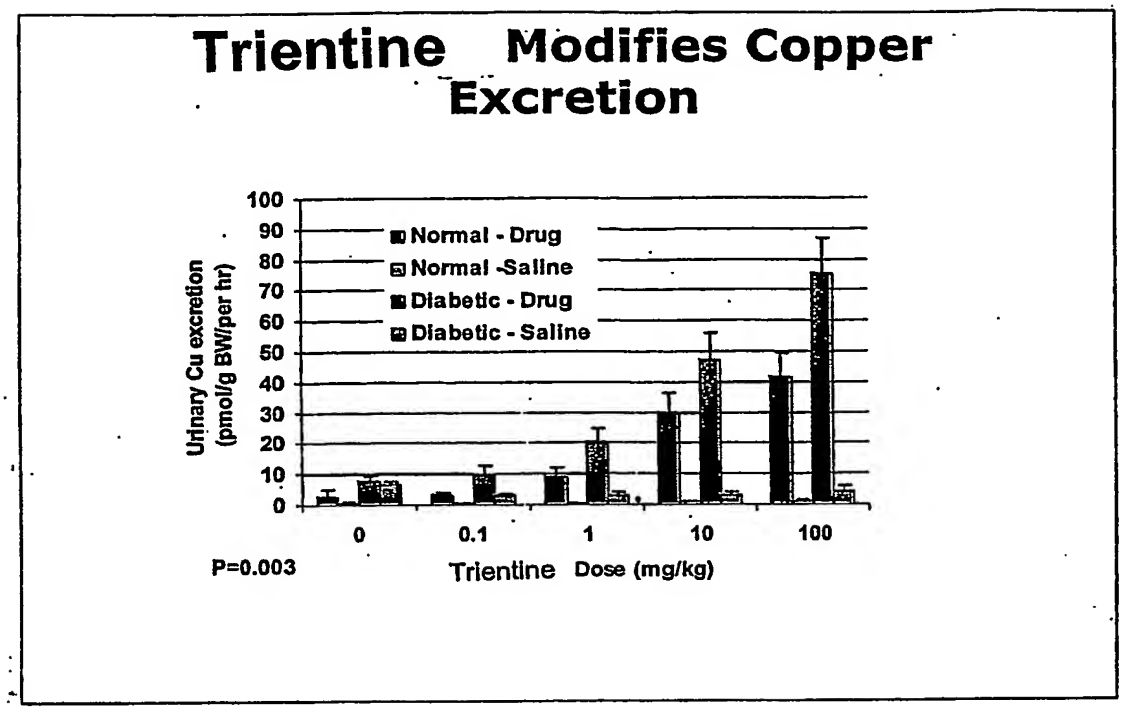


FIGURE 2B



No effect on iron excretion

Indicate where human dose is on x axis

FIGURE 3

Figure 4 Absolute Weight Change with time

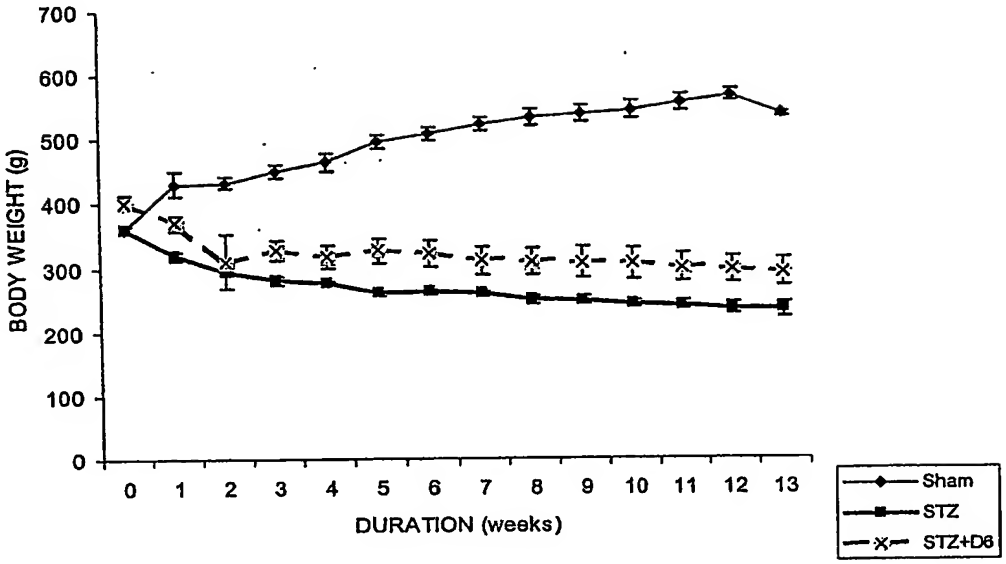


Figure 5 Percentage change in weight over time

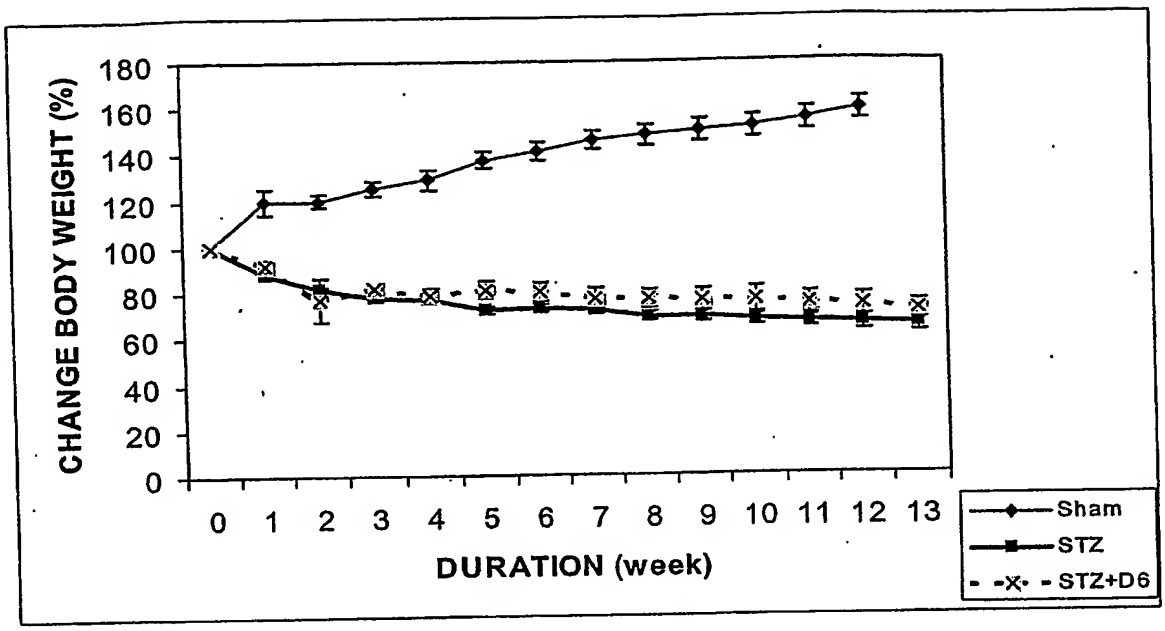


Figure 6 Blood Glucose change over time

(Weekly glucose measurements)

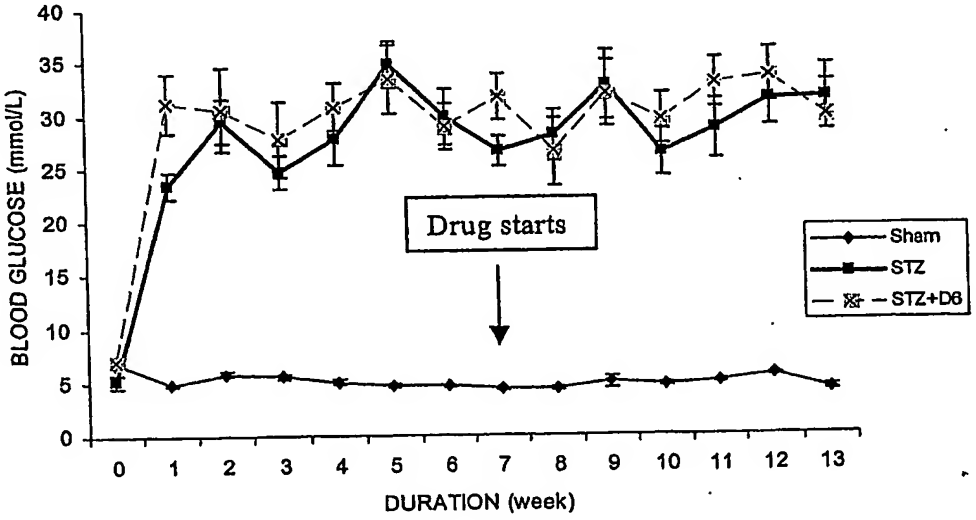


Figure 7 Cardiac output in response to increasing preload

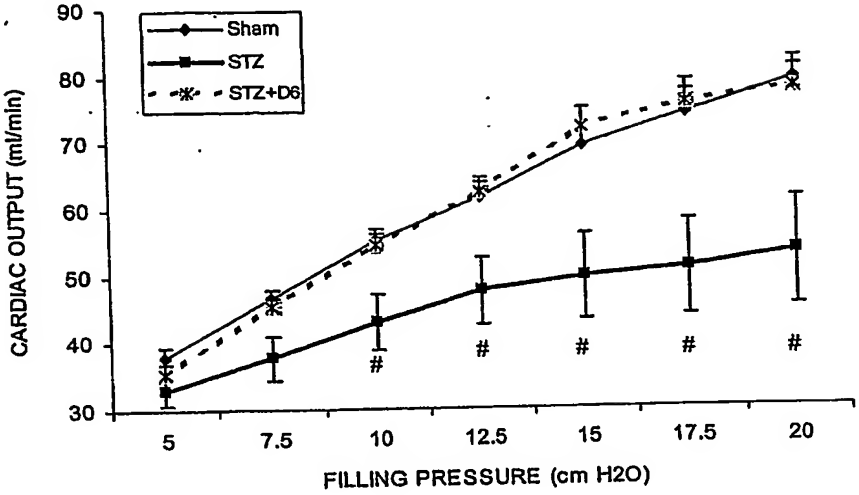


Figure 8. Absolute coronary flow in response to increasing preload

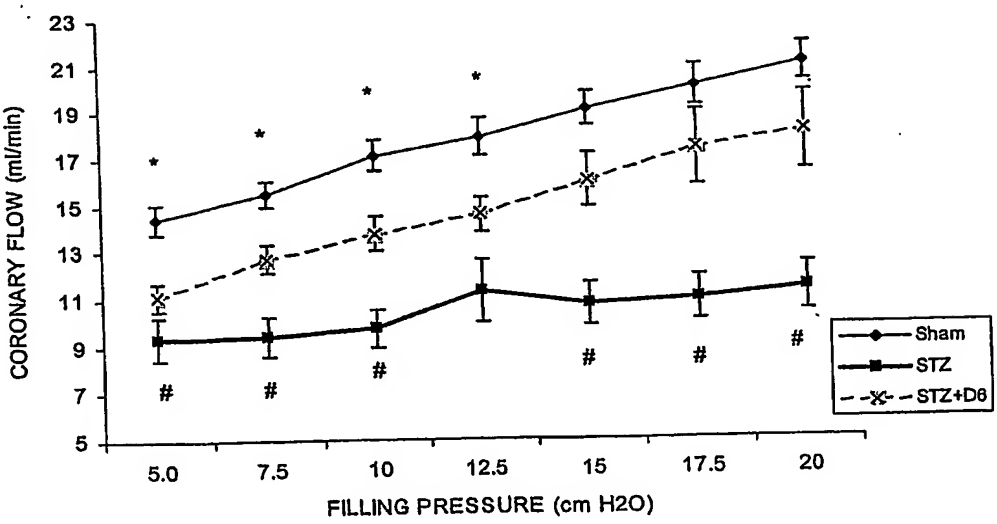


Figure 8A Coronary flow normalized to final cardiac weight

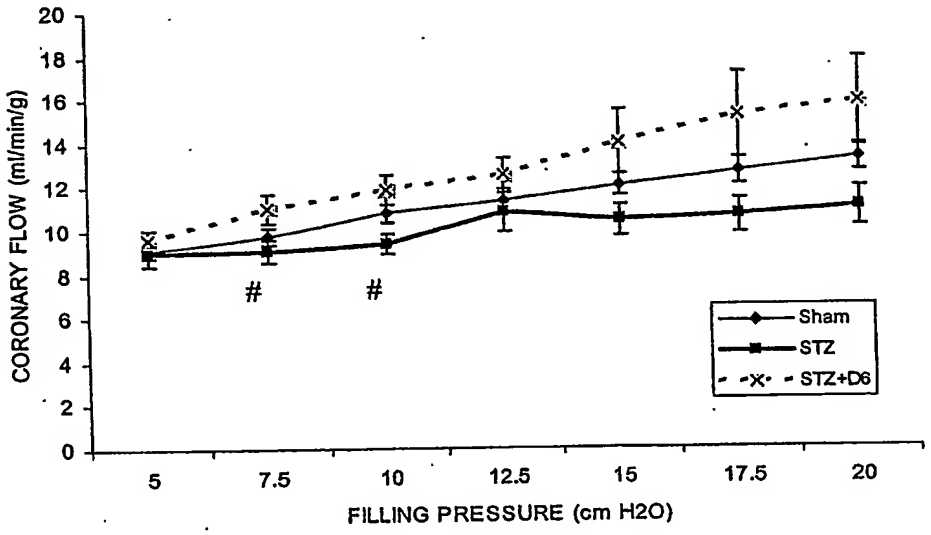


Figure 9 Aortic flow with increasing preload

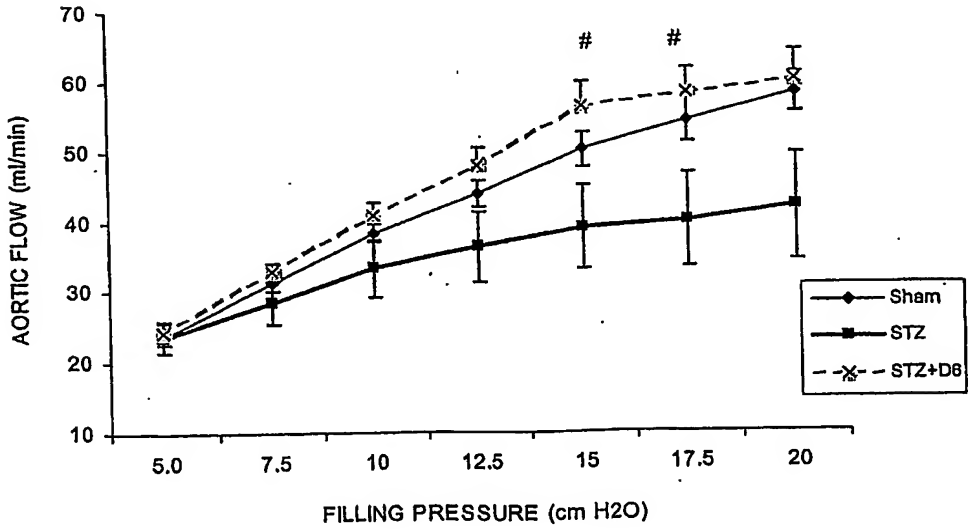


Figure 10 Mean left ventricular developed pressure (MLVDP) in response to increasing preload

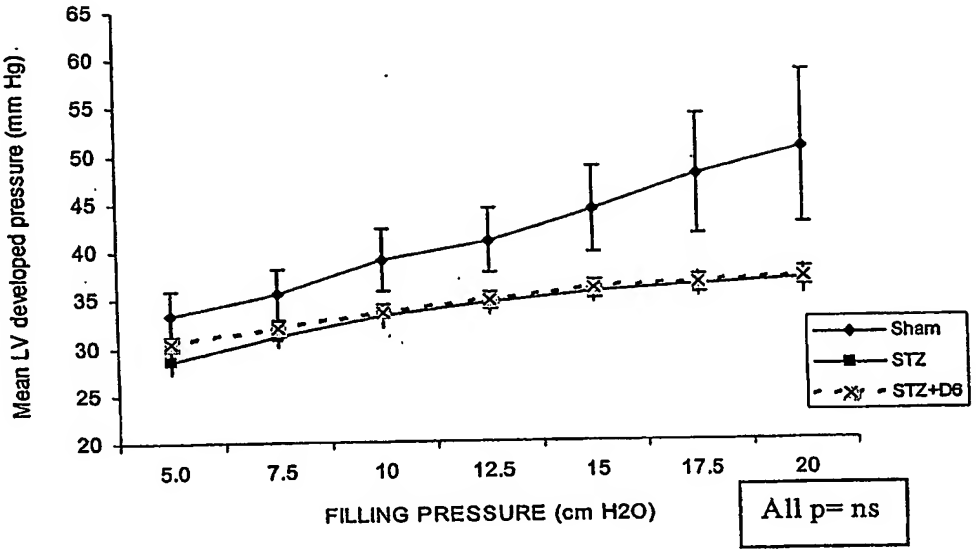


Figure 11 Maximal rate of positive change in ventricular pressure (contraction) in response to increasing preload

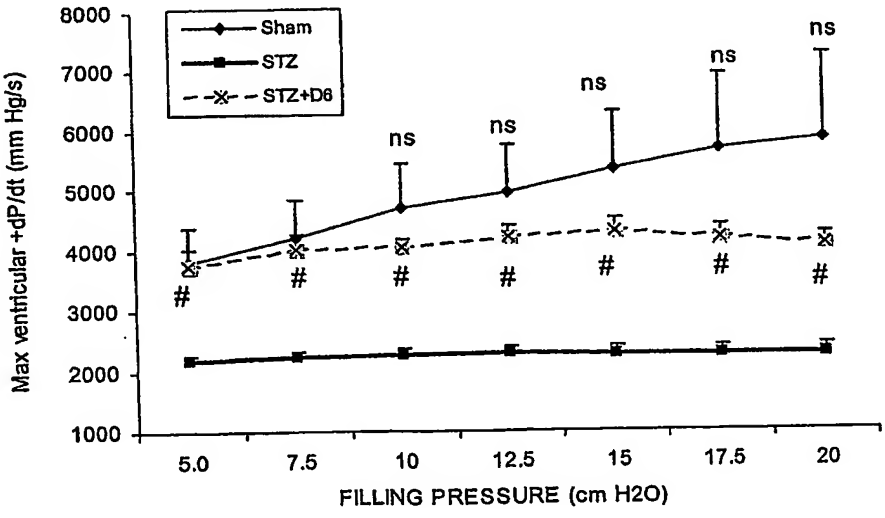


Figure 12 Maximal rate of decrease in ventricular pressure (relaxation) in response to increasing preload

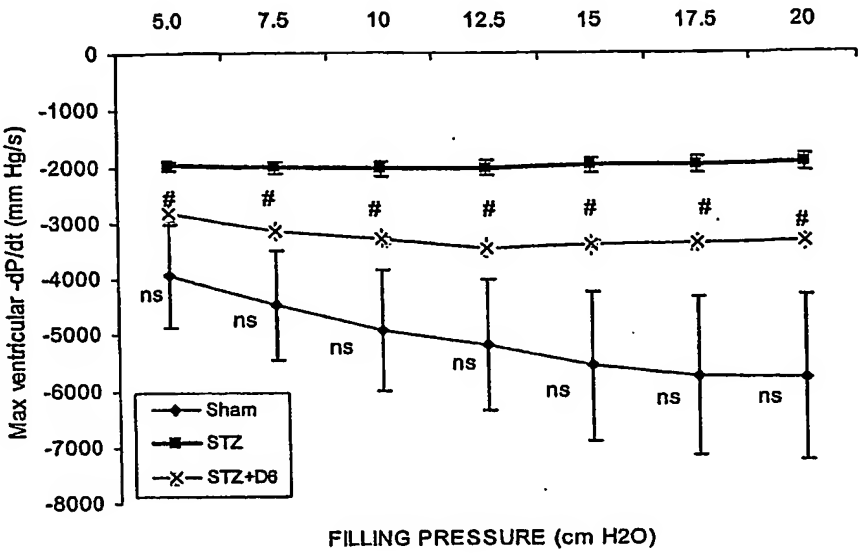


Figure 13 Maximal rate of positive change in aortic pressure in response to increasing preload

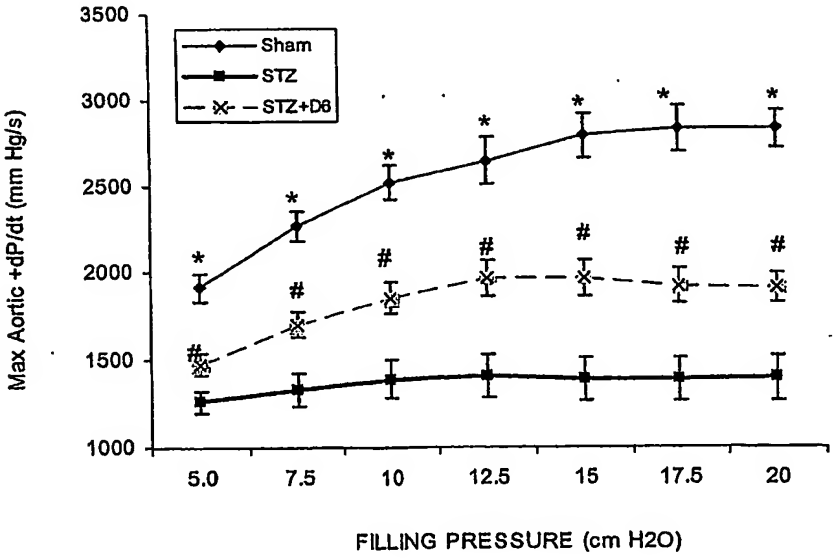


Figure 14 Maximal rate of decrease in aortic pressure in response to increasing preload

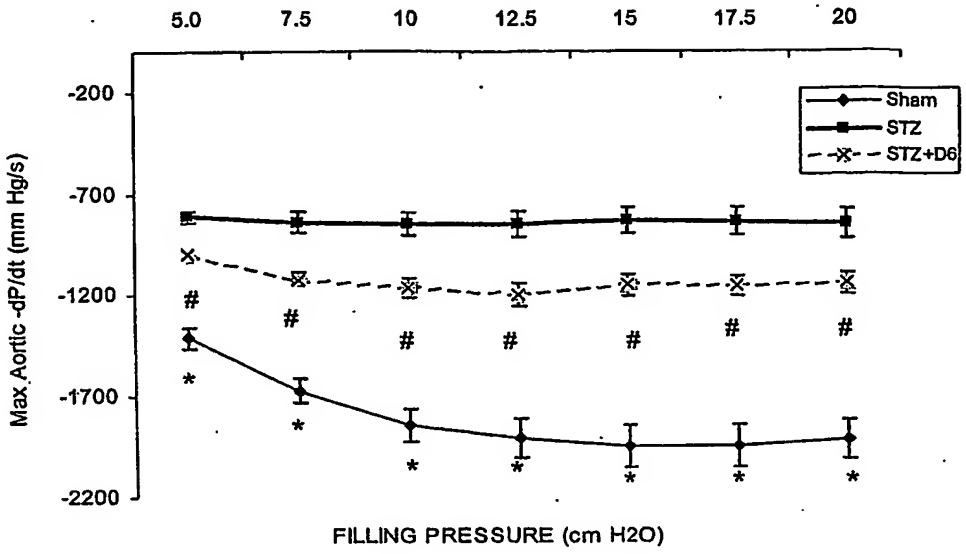


Figure 15 Percentage of functionally surviving hearts at each afterload pressure

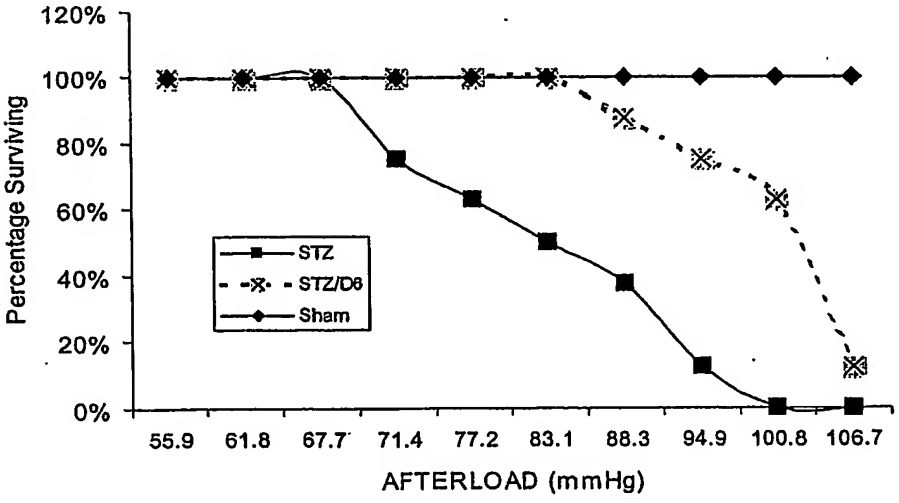


Figure 16 Cardiac output in response to increasing afterload

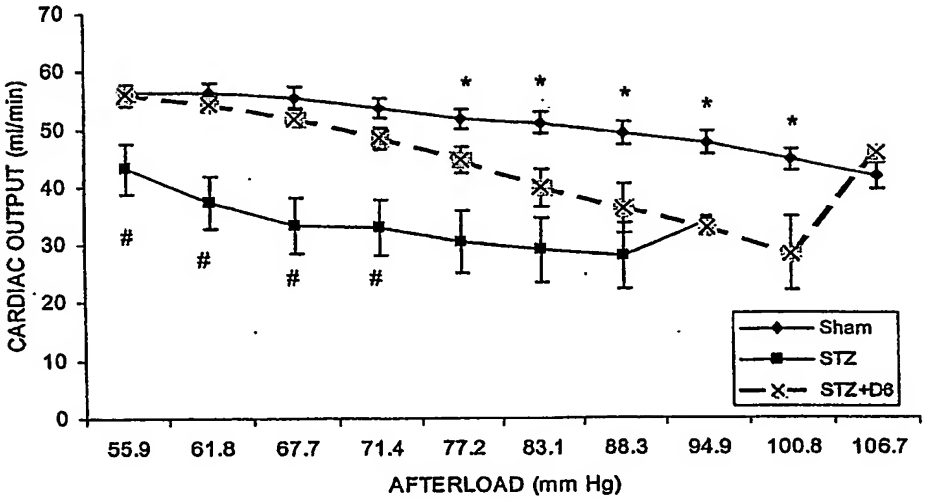


Figure 17A Absolute change in coronary flow in response to increasing afterload

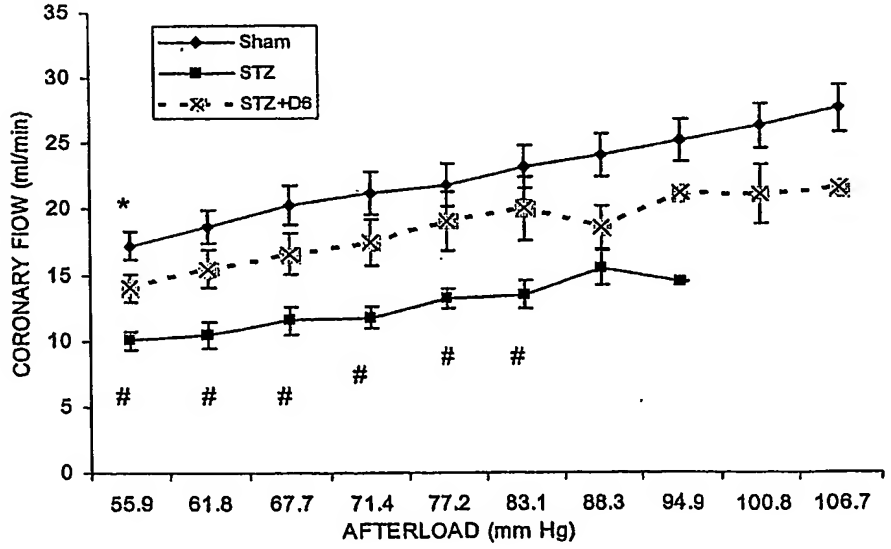


Figure 17B Change in coronary flow in response to increasing afterload normalized to heart weight

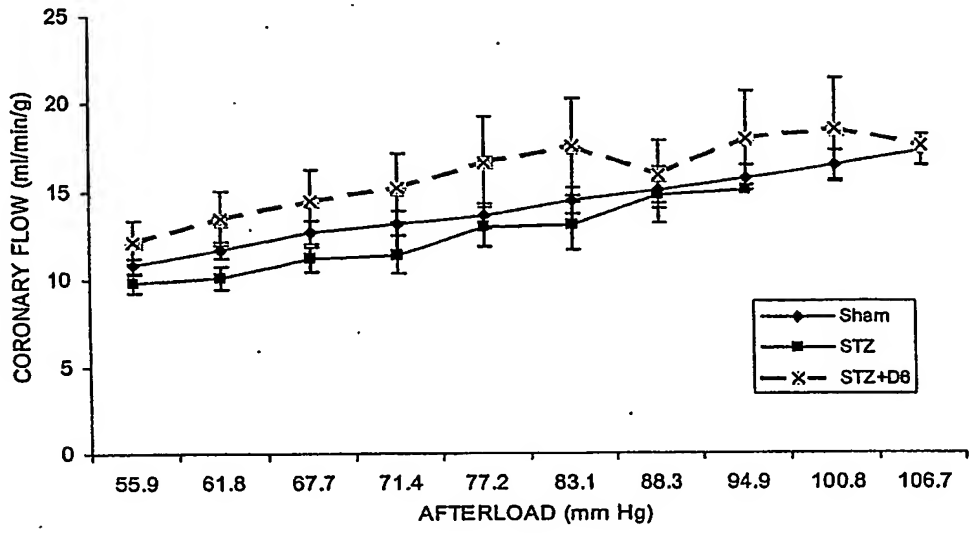


Figure 18 Mean left ventricular developed pressure (MLVDP) in response to increasing afterload

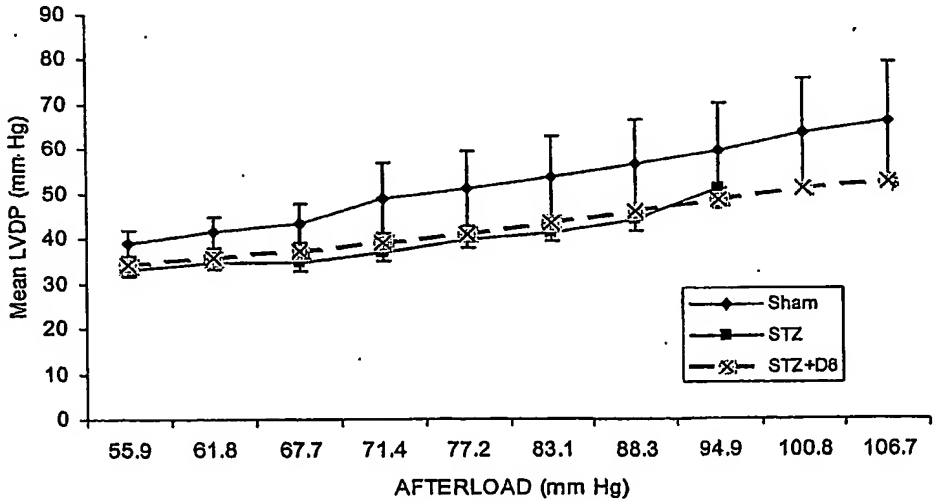


Figure 19. Maximal rate of positive change in ventricular pressure (contraction) in response to increasing afterload

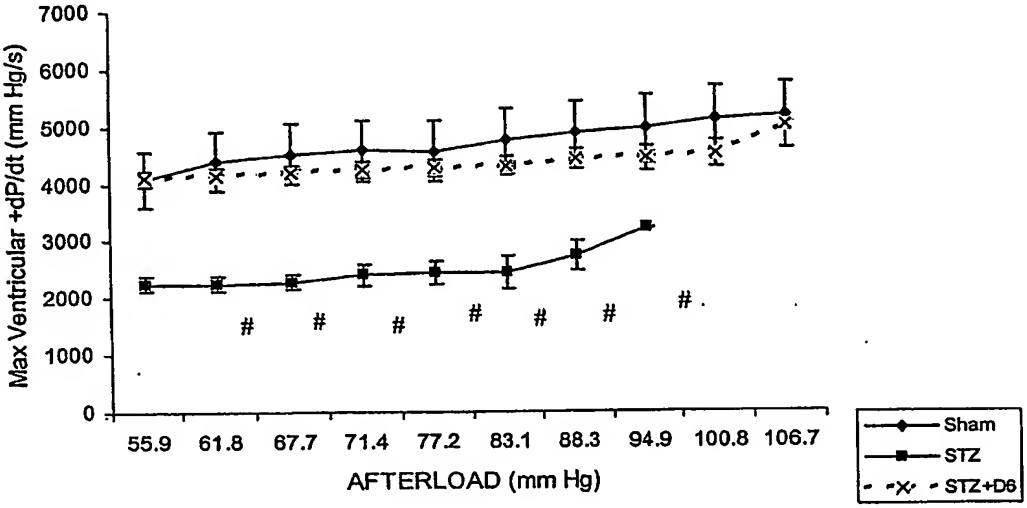


Figure 20. Maximal rate of decrease in ventricular pressure (relaxation) in response to increasing afterload

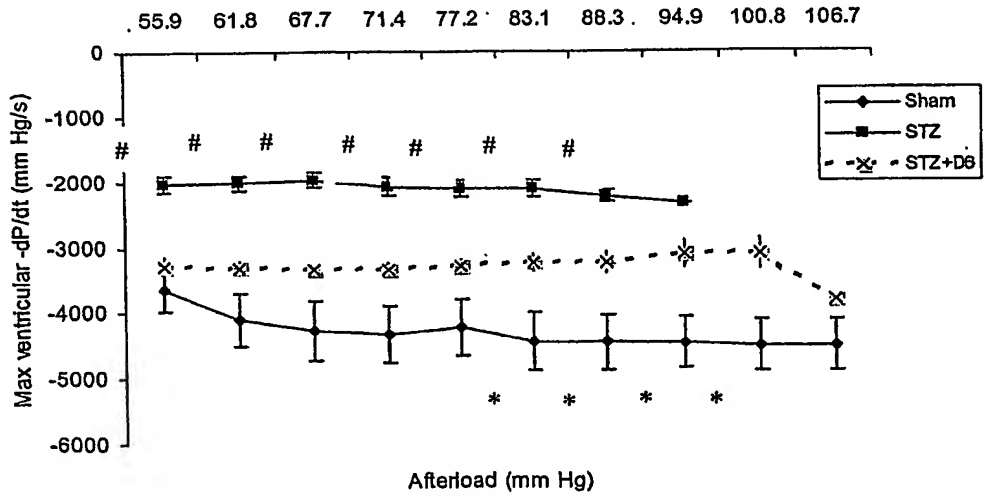


Figure 21 Maximal rate of positive change in aortic pressure in response to increasing afterload

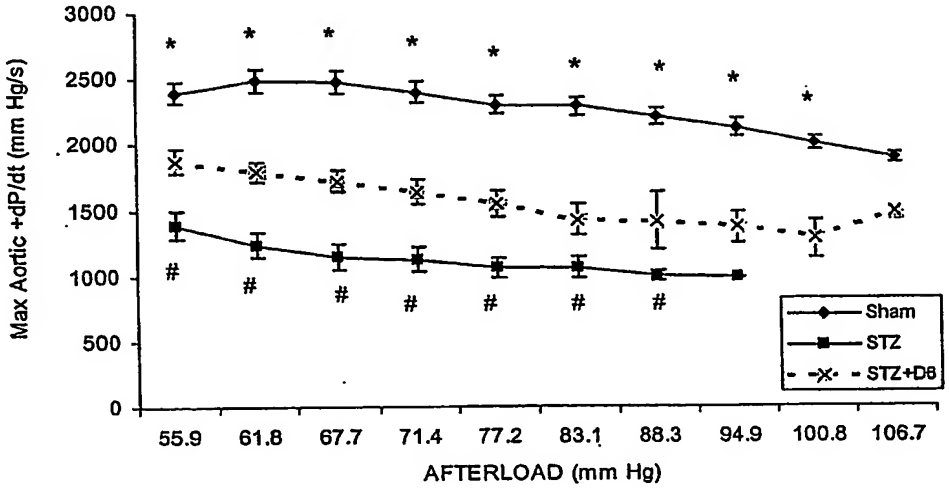


Figure 22. Maximal rate of decrease in aortic pressure in response to increasing afterload

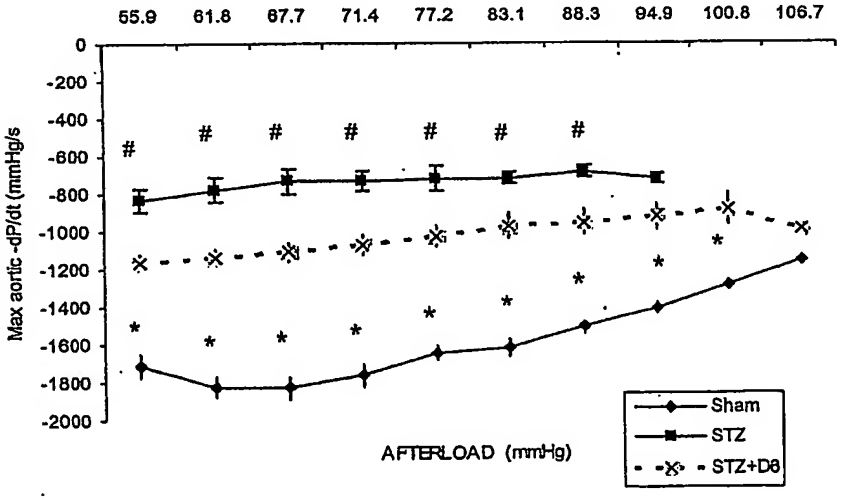
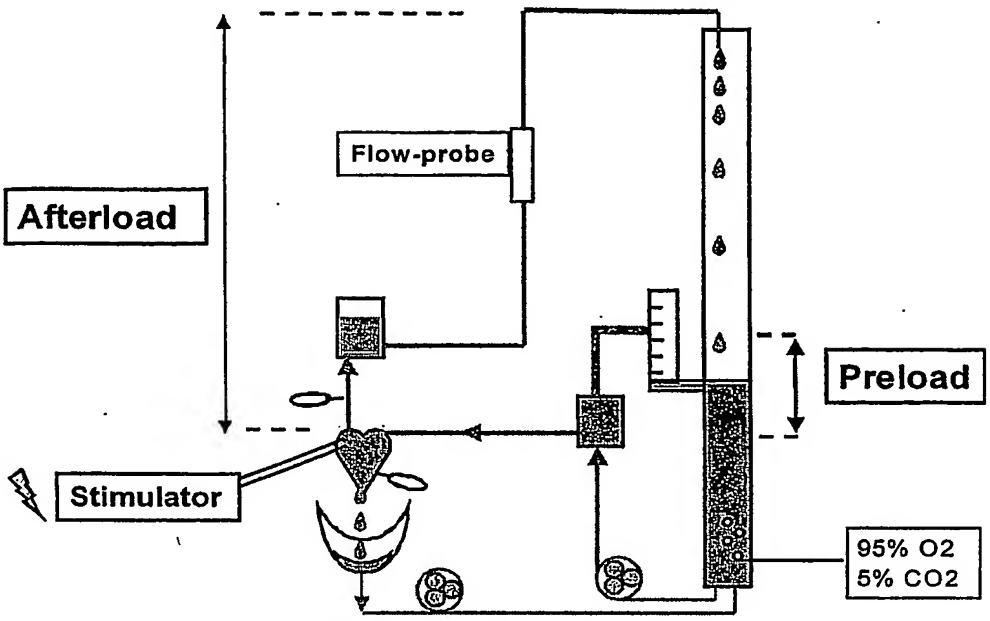
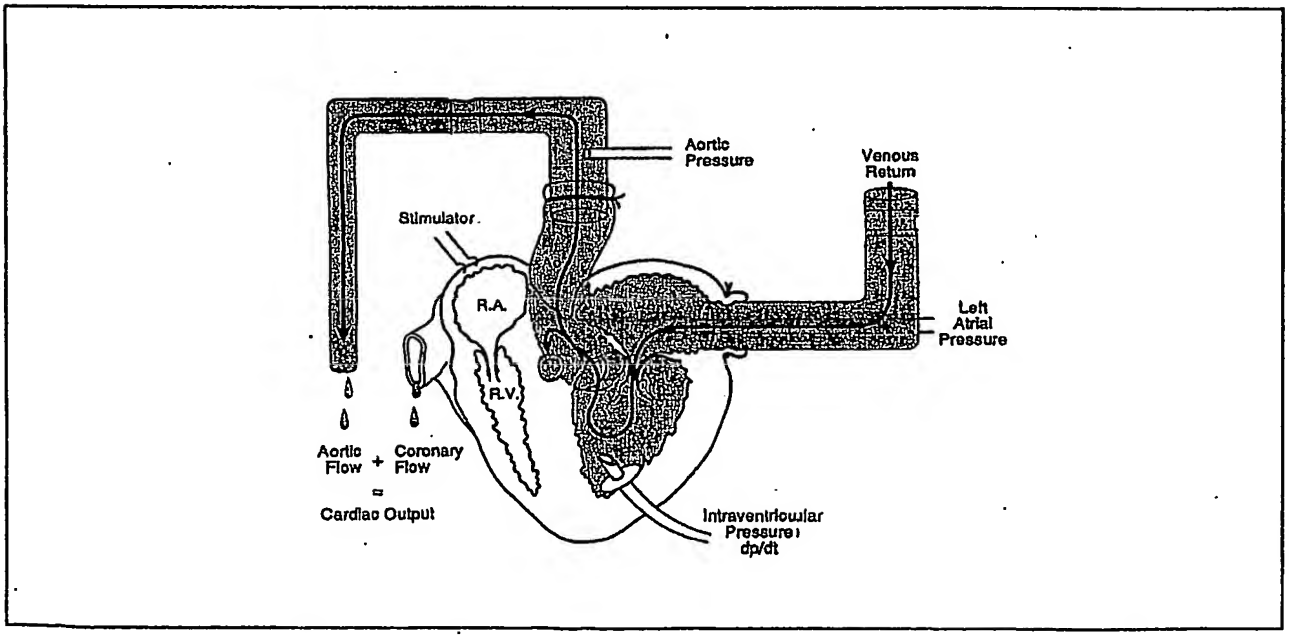


Figure 23A Apparatus for working heart



Pressure transducers

Figure 23B Heart in more detail



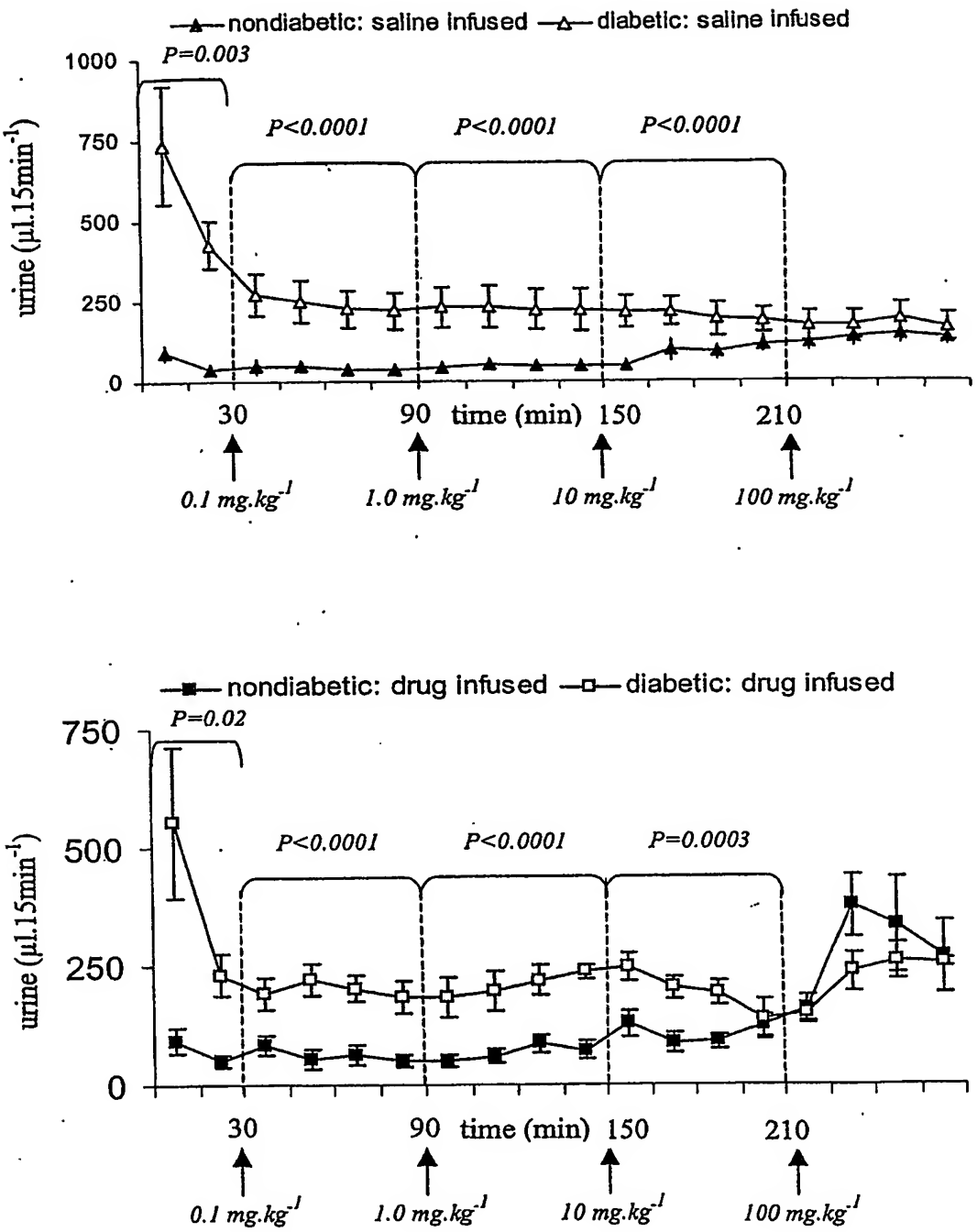


Fig. 24. Urine excretion in diabetic and nondiabetic animals in response to increasing doses of trientine (*bottom;* 0.1, 1.0, 10, 100 mg.kg^{-1} in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline *(top)*. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

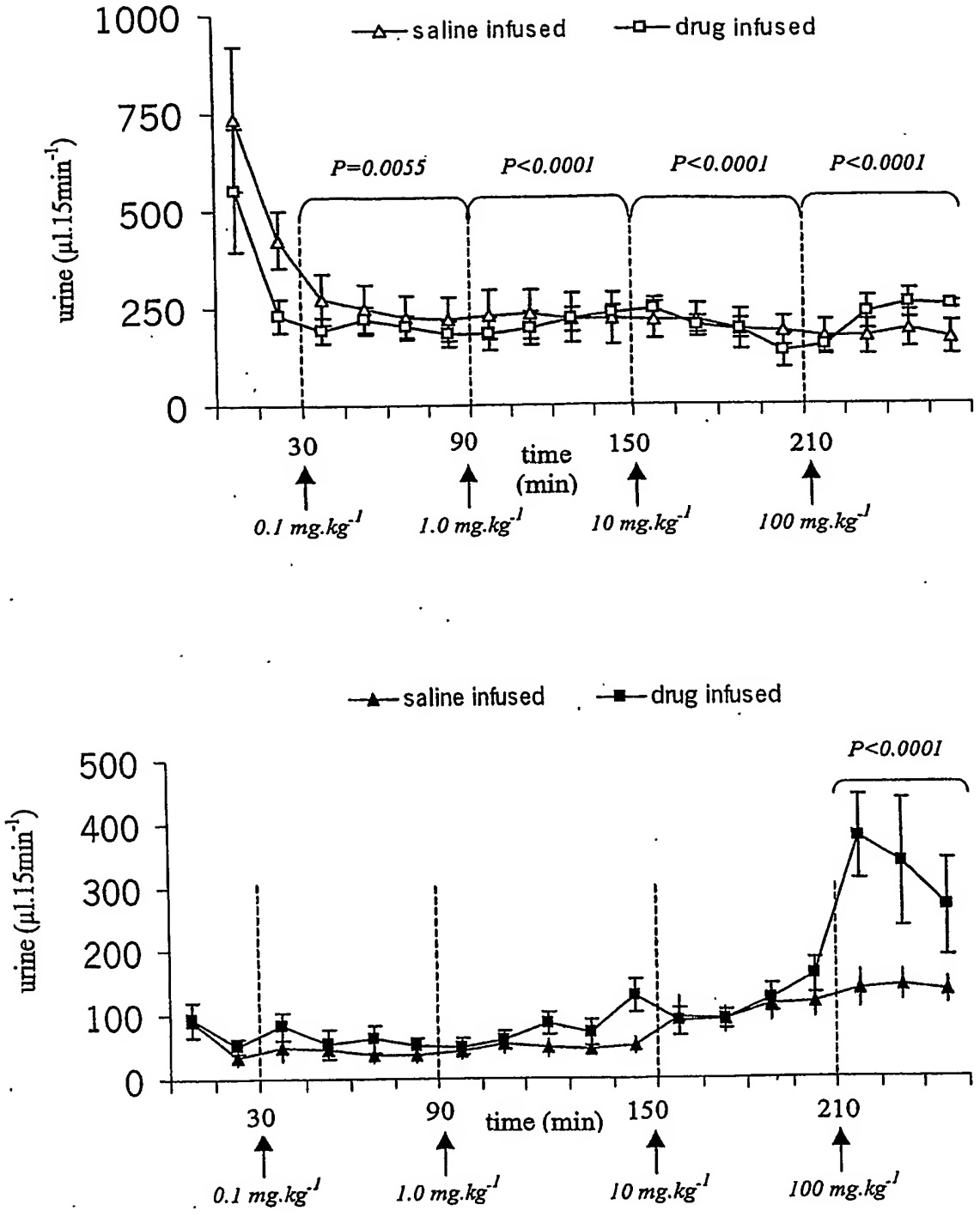


Fig. 25. Urine excretion in diabetic *(top)* and nondiabetic *(bottom)* rats receiving increasing doses of trientine (0.1, 1.0, 10, 100 mg.kg^{-1} in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

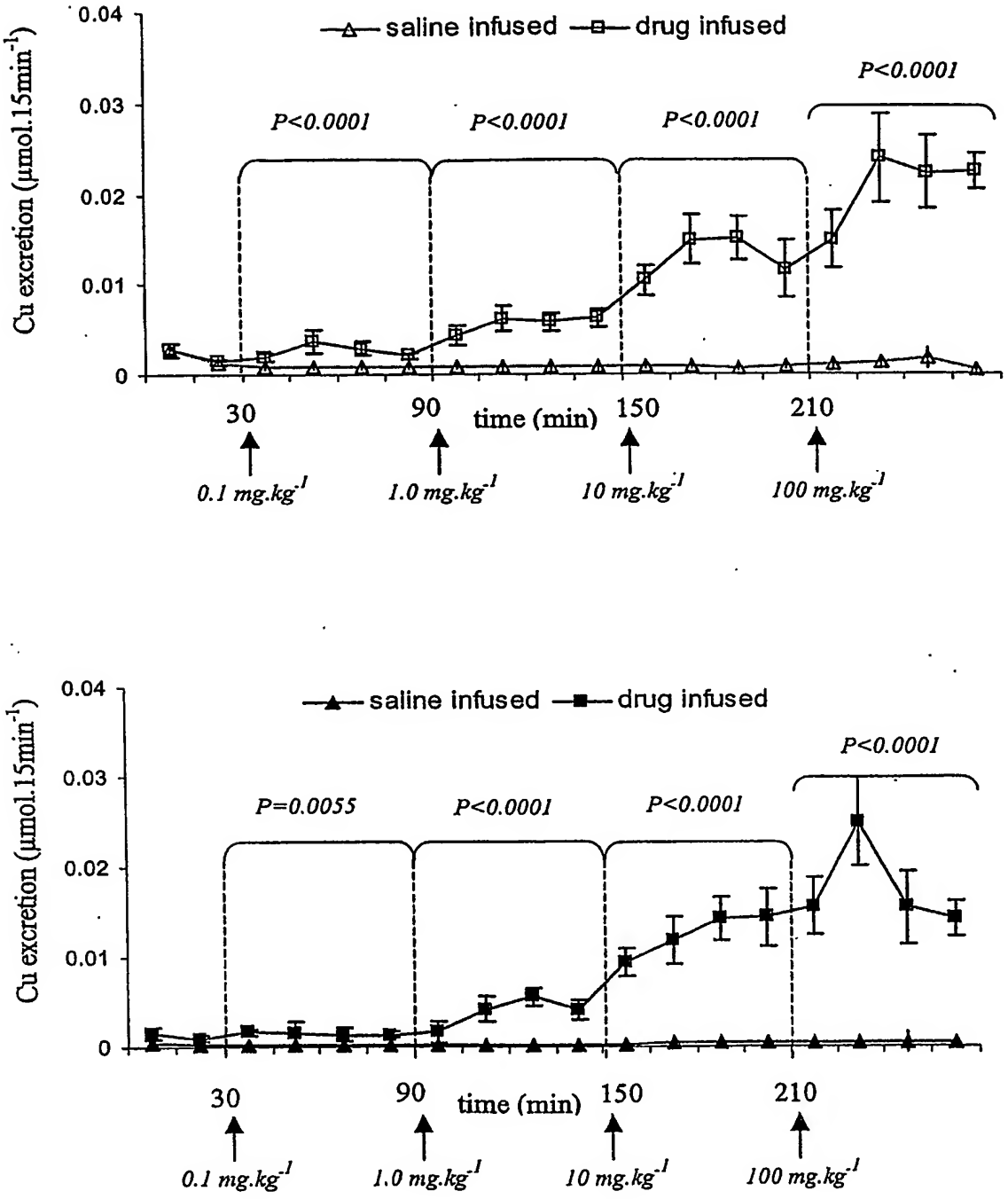


Fig. 26. Copper excretion in urine of diabetic *(top)* and nondiabetic *(bottom)* rats receiving increasing doses of trientine (0.1, 1.0, 10, 100 $mg.kg^{-1}$ in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

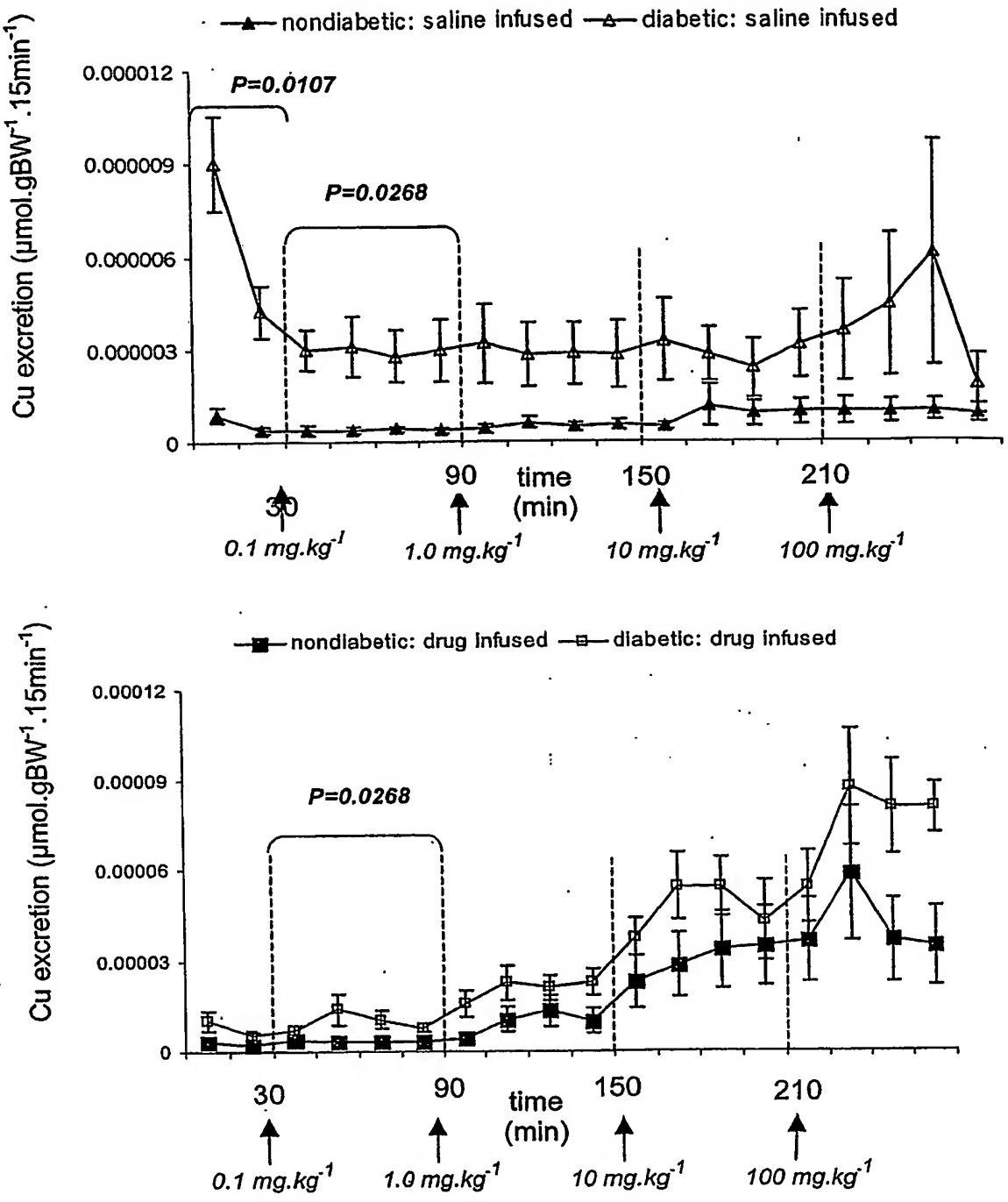


Fig. 27. Urinary copper excretion per gram of bodyweight in diabetic and nondiabetic animals in response to increasing doses of trientine (*bottom;* 0.1, 1.0, 10, 100 mg.kg^{-1} in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline *(top)*. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

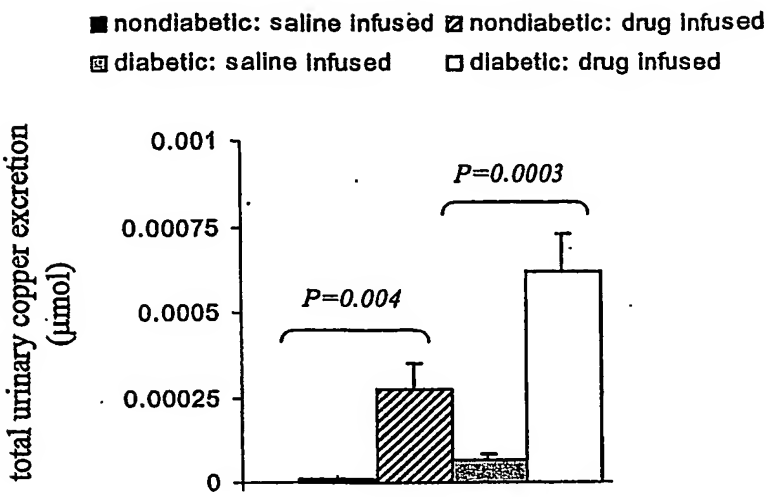


Fig. 28. Total urinary copper excretion (µmol) in nondiabetic animals administered saline (black bar, n = 7) or trientine (hatched bar, n = 7) and in diabetic animals administered saline (grey bar, n = 7) or trientine (white bar, n = 7). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

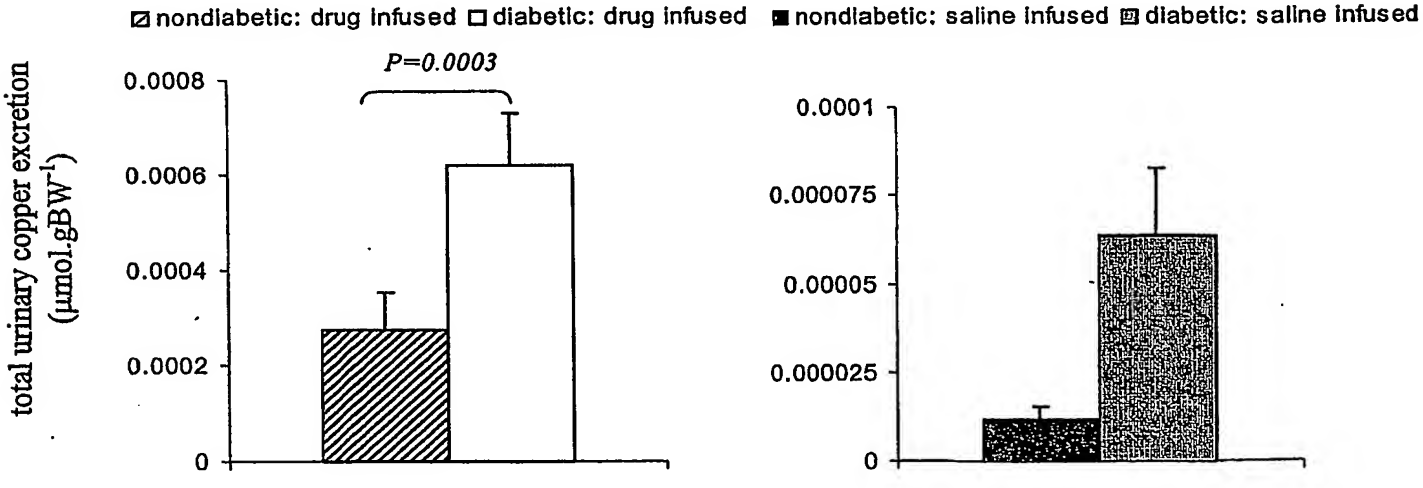


Fig. 29. Total urinary copper excretion per gram of bodyweight ($\mu g.gBW^{-1}$) in animals receiving trientine (nondiabetic: hatched bar, n = 7; diabetic: white bar, n = 7) or saline (nondiabetic: black bar, n = 7; diabetic: grey bar, n = 7). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

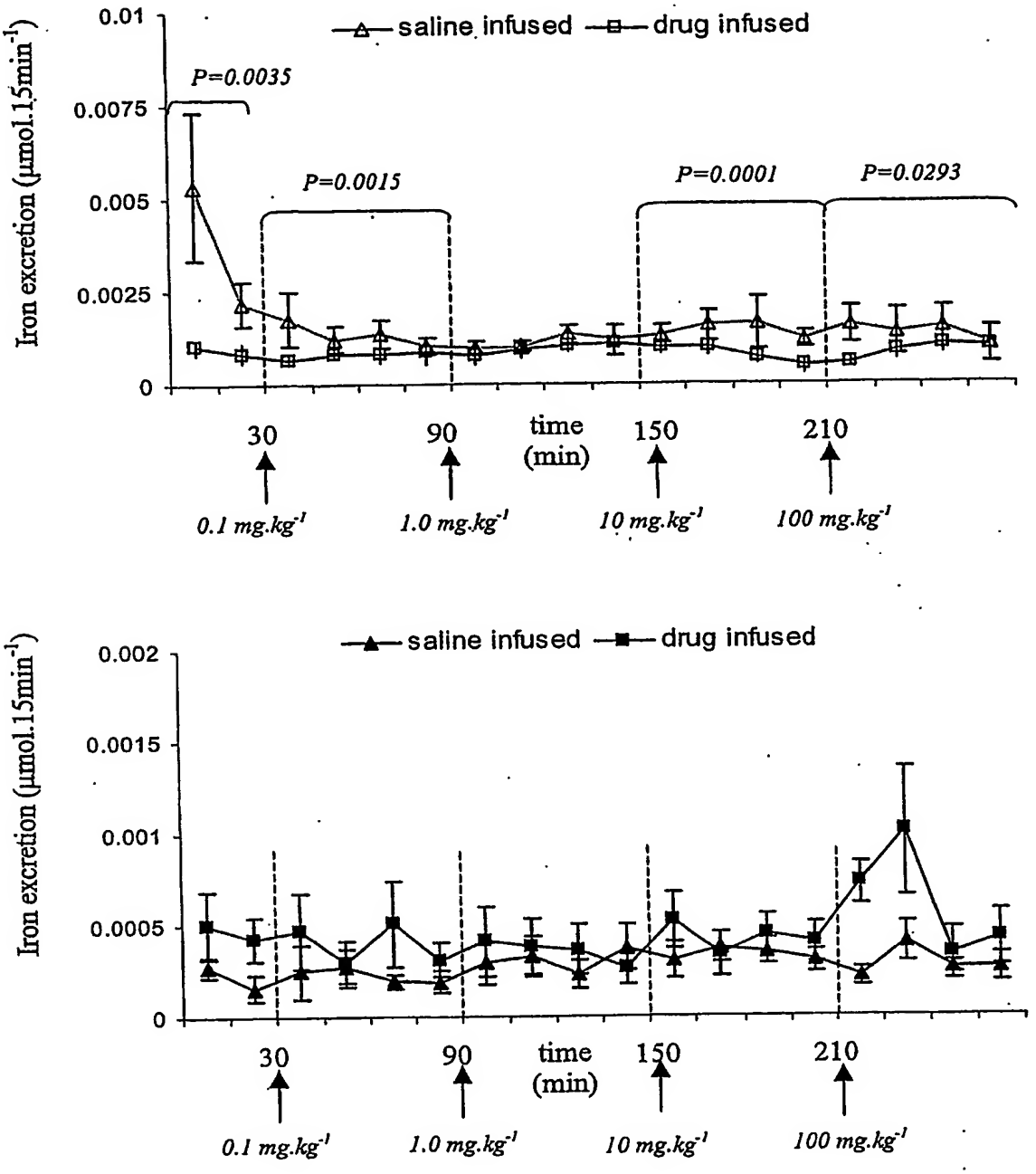


Fig. 30. Iron excretion in urine of diabetic *(top)* and nondiabetic *(bottom)* rats receiving increasing doses of trientine (0.1, 1.0, 10, 100 mg.kg^{-1} in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

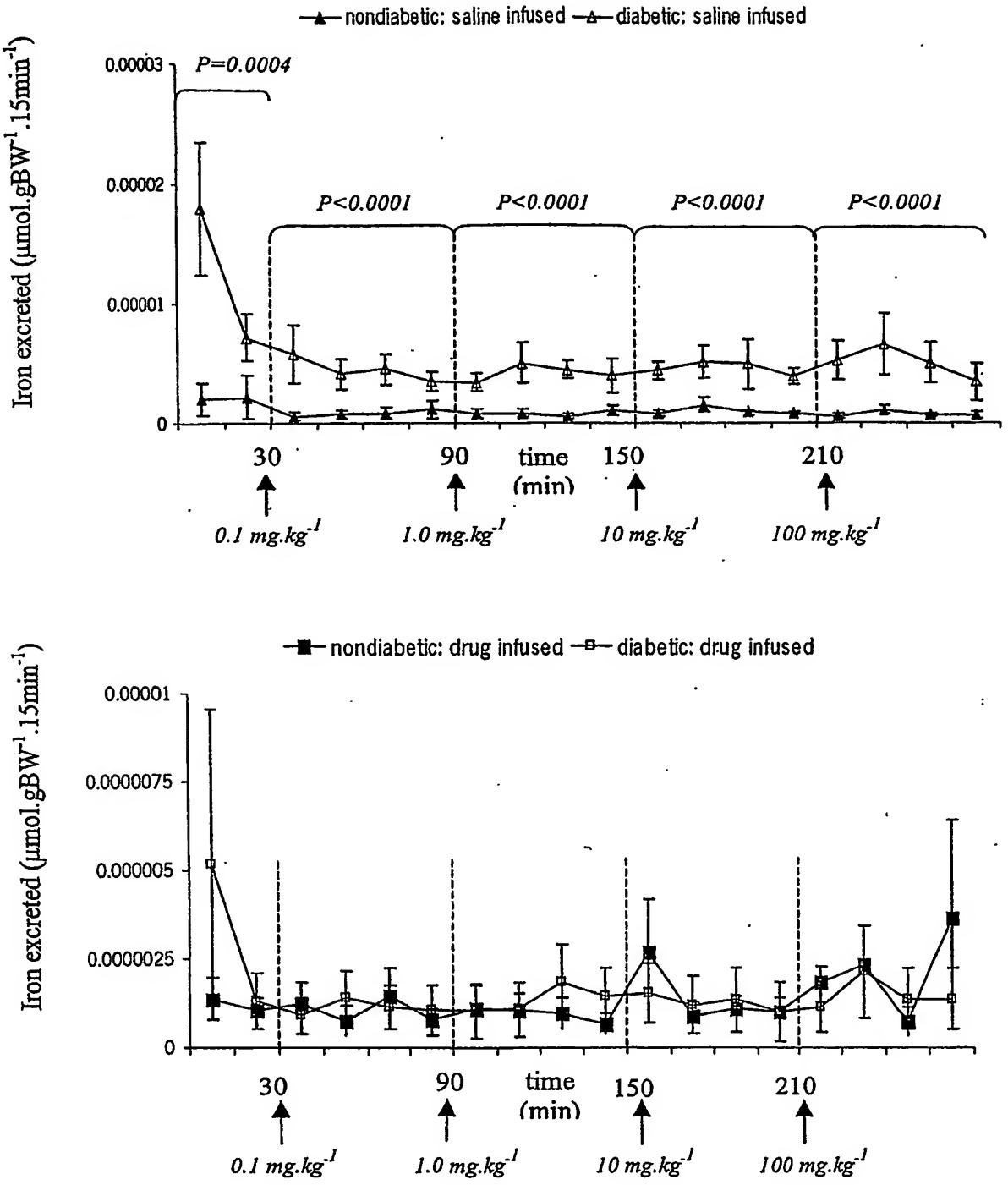


Fig. 31. Urinary iron excretion per gram of bodyweight in diabetic and nondiabetic animals in response to increasing doses of trientine (*bottom;* 0.1, 1.0, 10, 100 mg.kg^{-1} in 75 µl saline followed by 125 µl saline flush injected at time shown by arrow) or an equivalent volume of saline *(top)*. Each point represents a 15 min urine collection period (see Methods for details). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

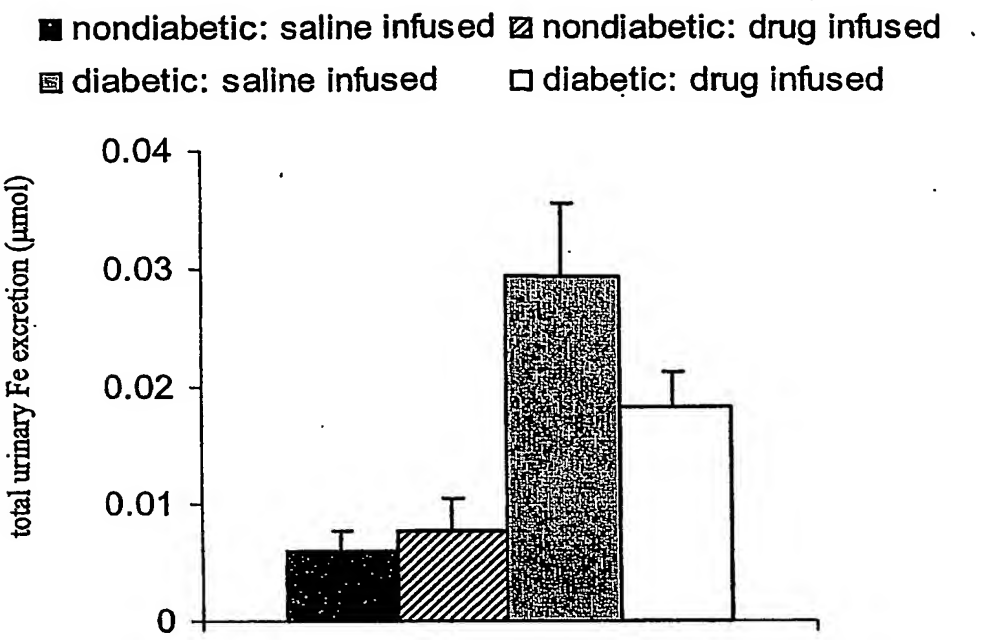


Fig. 32. Total urinary iron excretion (µmol) in nondiabetic animals administered saline (black bar, n = 7) or trientine (hatched bar, n = 7) and in diabetic animals administered saline (grey bar, n = 7) or trientine (white bar, n = 7). Error bars show SEM and *P* values are stated if significant ($P < 0.05$).

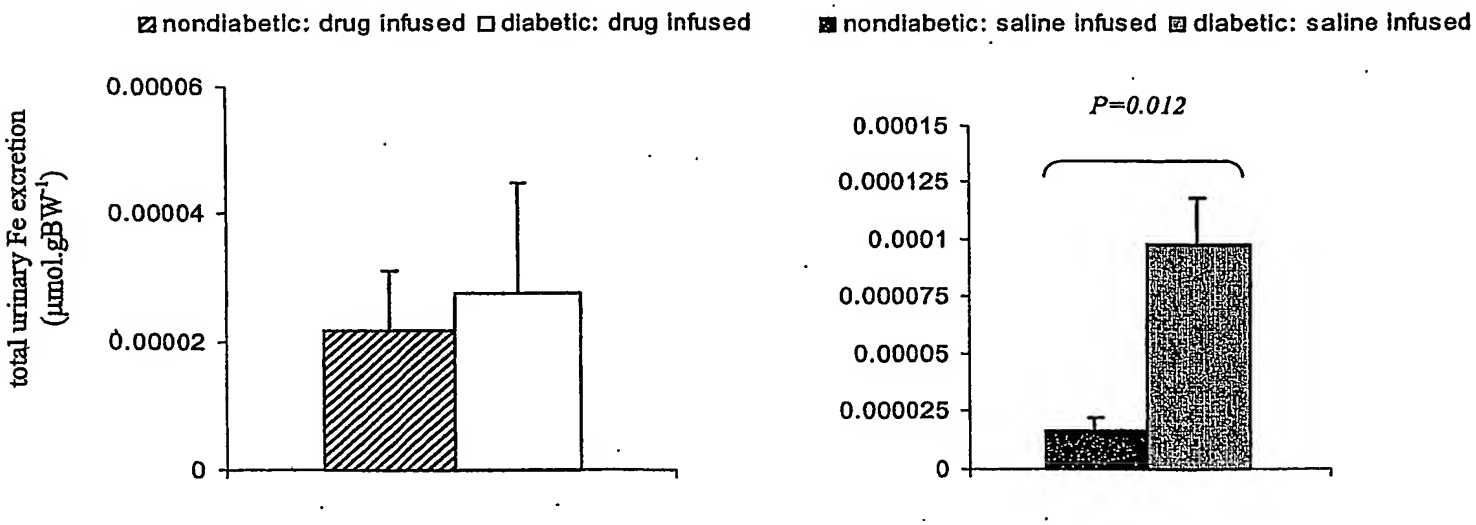


Fig. 33. Total urinary iron excretion per gram of bodyweight ($\mu g.gBW^{-1}$) in animals receiving trientine (nondiabetic: hatched bar, n = 7; diabetic: white bar, n = 7) or saline (nondiabetic: black bar, n = 7; diabetic: grey bar, n = 7). Error bars show SEM and *P* values are stated if significant ($P \leq 0.05$).

Figure 34 Percentage of surviving hearts at each afterload pressure

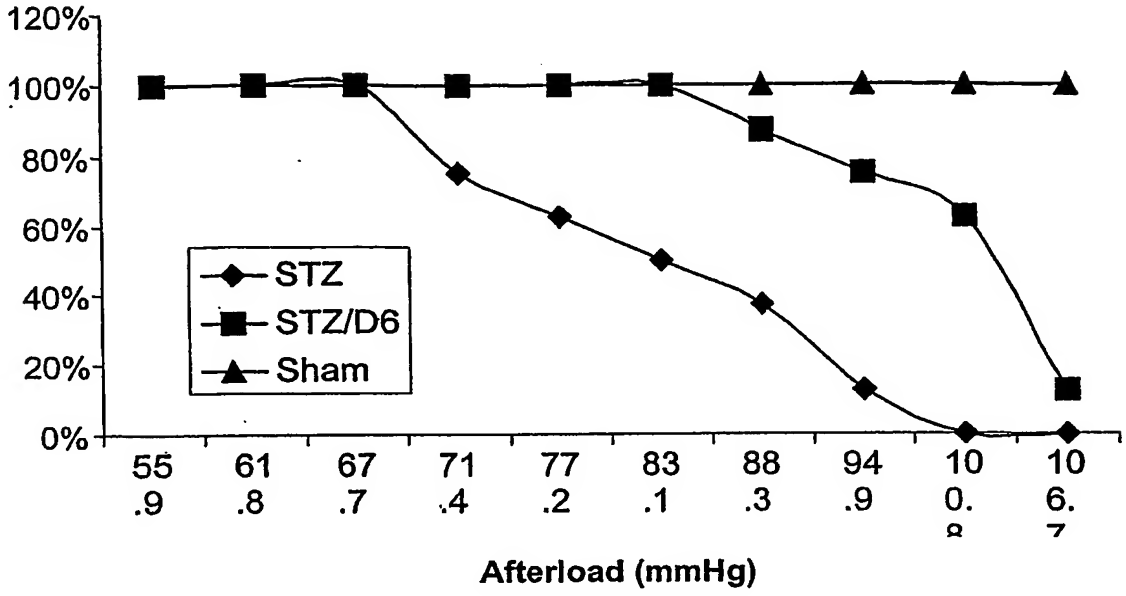


FIGURE 35

Cu excretion			Dose level		
Mixed Model Effects	**Baseline**	**0.1 mg.kg^{-1}**	**1.0 mg.kg^{-1}**	**10 mg.kg^{-1}**	**100 mg.kg^{-1}**
Diabetes	$F_{1,24}$ = 18.52	$F_{1,24}$ = 19.82	$F_{1,24}$ = 21.92	$F_{1,24}$ = 9.93	$F_{1,24}$ = 17.82
(normal/diabetic rats)	P = 0.0002	P = 0.0002	P < 0.0001	P < 0.0001	P < 0.0003
Drug	$F_{1,24}$ = 1.73	$F_{1,24}$ = 24.94	$F_{1,24}$ = 78.36	$F_{1,24}$ = 135.36	$F_{1,24}$ = 162.17
(drug/saline)	*NS*	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Interaction	$F_{1,24}$ = 0.16	$F_{1,24}$ = 3.58	$F_{1,24}$ = 7.16	$F_{1,24}$ = 6.02	$F_{1,24}$ = 12.43
	NS	*NS*	P < 0.0132	P < 0.0218	P < 0.0017
Sampling time (repeated measure)	t_1, t_2	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4

Fe excretion			Dose level		
Mixed Model Effects	**Baseline**	**0.1 mg.kg^{-1}**	**1.0 mg.kg^{-1}**	**10 mg.kg^{-1}**	**100 mg.kg^{-1}**
Diabetes	$F_{1,23}$ = 12.87	$F_{1,23}$ = 15.82	$F_{1,24}$ = 22.68	$F_{1,24}$ = 14.93	$F_{1,24}$ = 7.35
(normal/diabetic rats)	P = 0.0016	P = 0.0006	P < 0.0001	P = 0.0007	P = 0.0122
Drug	$F_{1,23}$ = 8.6	$F_{1,23}$ = 7.89	$F_{1,24}$ = 12.23	$F_{1,24}$ = 10.91	$F_{1,24}$ = 2.47
(drug/saline)	P = 0.0075	P = 0.01	P < 0.0019	P = 0.003	P = 0.1292
Interaction	$F_{1,23}$ = 12.10	$F_{1,23}$ = 15.06	$F_{1,24}$ = 14.07	$F_{1,24}$ = 17.72	$F_{1,24}$ = 16.76
	P = 0.002	P = 0.0008	P = 0.001	P = 0.0003	P = 0.0004
Sampling time (repeated measure)	2	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4	t_1, t_2, t_3, t_4

Plasma concentration-time profiles of trientine after oral administration to four male patients[1]

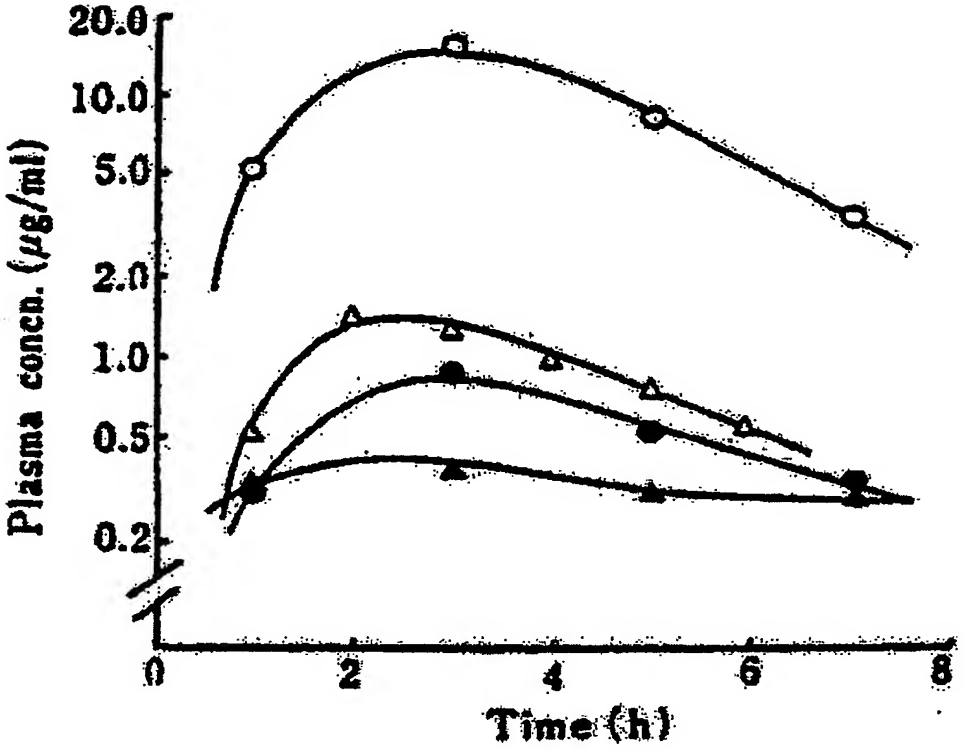


FIGURE 36

Plasma concentration-time profiles of trientine after oral administration to four female patients[1]

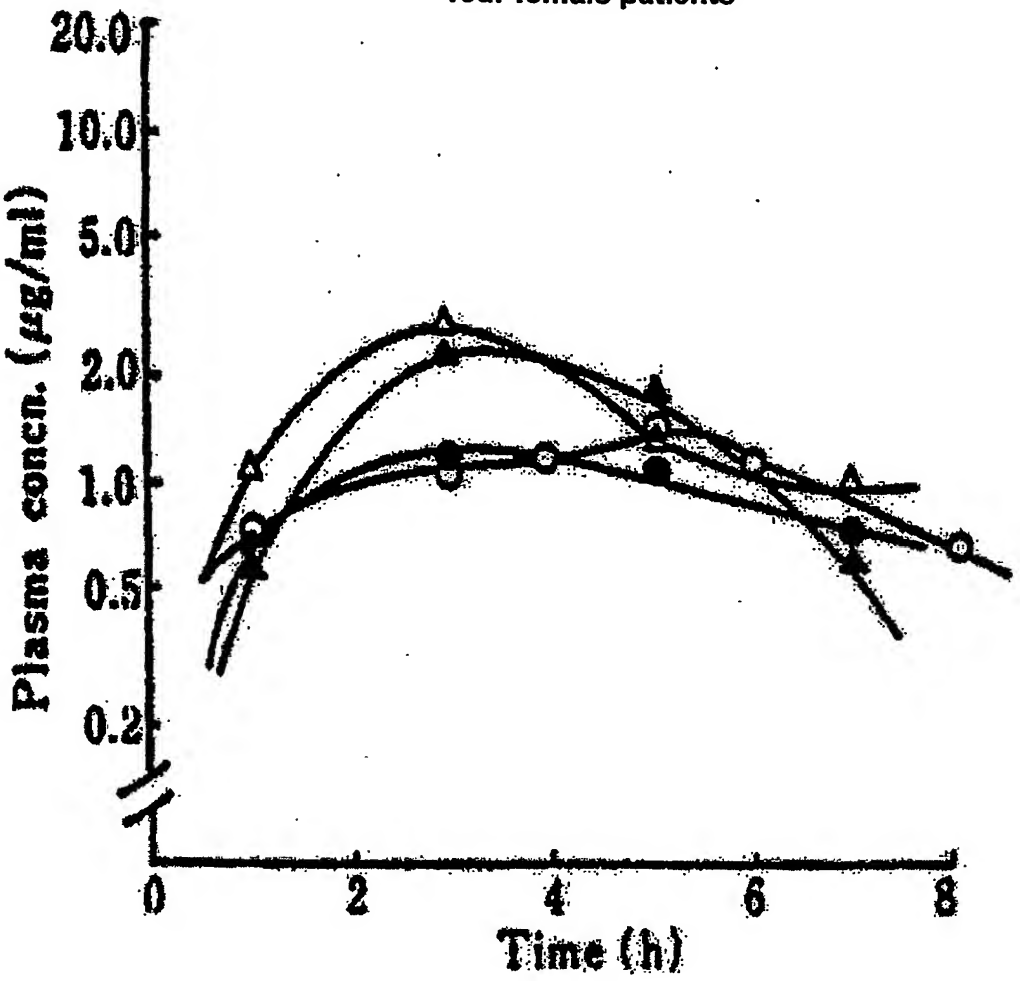


FIGURE 37